



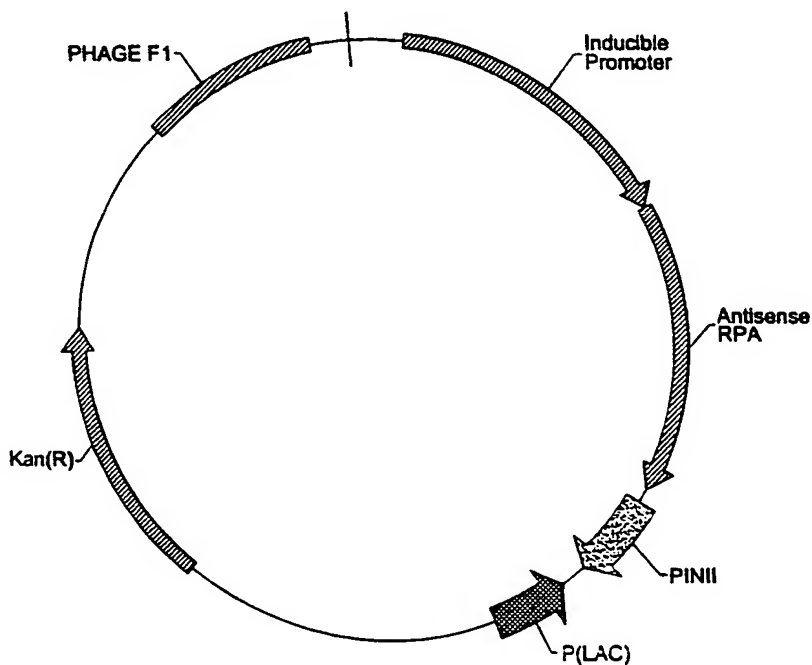
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(54) Title: MAIZE REPLICATION PROTEIN A



(57) Abstract

Methods and compositions for modulating DNA metabolism are provided. Nucleotide and amino acid sequences encoding a maize replication protein A subunit are provided. The sequences can be used in expression cassettes for modulating DNA replication, DNA repair, and recombination.

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MAIZE REPLICATION PROTEIN A

FIELD OF THE INVENTION

The invention relates to the genetic manipulation of plants, particularly to modulating DNA metabolism in transformed plants and plant cells.

5 BACKGROUND OF THE INVENTION

Replication protein A (RPA) is a single-stranded DNA-binding protein that is required for multiple processes in eukaryotic cells. RPA from human cells is a stable complex of 70-, 32-, and 14-kDa subunits. Homologues of RPA have been identified in all eukaryotes examined. However, only human RPA and closely
10 related homologues can support SV40 DNA replication.

The RPA complex appears to be highly conserved in all eukaryotes. The three RPA genes in budding yeast cells are essential for cell viability. Nevertheless, yeast RPA only partially substitutes for human RPA in the *in vitro* replication of simian virus 40 indicating that species-specific interactions between
15 RPA and other replication proteins may be important for its biological activity.

RPA binds tightly to single stranded DNA as a heterotrimeric complex. The binding activity has been localized to the 70 kDa subunit. The affinity of RPA for both double-stranded DNA and RNA is at least three orders of magnitude lower than it is for single-stranded DNA. It has been reported that RPA binds
20 preferentially to the pyrimidine-rich strand of both *S. cerevisiae* sequences and the SV40 origin of replication. However, studies examining the determinants of replication origins in *S. cerevisiae* indicate that this preferential binding is not critical for the initiation of DNA replication.

25 Subunits of RPA in the 70-, 32- and 14 kDa ranges have been identified from various sources. The 32kDa subunit has also been referred to as "RPA2", "B", "small", "32kDa", "P32", "P34", and "middle" subunit. For the purposes of this invention, the "middle" subunit is intended as the subunit having a molecular weight of about 32 kDa.

30 The middle subunit of RPA has a role in cell cycle regulation; single stranded DNA binding; affinity of DNA binding; species-specificity of DNA

binding; DNA recombination, repair, replication and metabolism; and response to DNA damages. (Anderson (1966) *Calif. Inst. Technol.*; Seroussi *et al.* (1993) *J. Biol. Chem.* 268:7147-54; Kenny *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:9757-61; Brush *et al.* (1995) *Methods Enzymol.* 262:522-48; Stigger *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:579-83; Philipova *et al.* (1996) *Genes Dev.* 10:2222-33).

Much research has centered on the exploration of the biochemical and genetic mechanisms by which cell cycle regulation of DNA synthesis is achieved. While there have been advances in delineating the existence of cell cycle proteins, more information is needed on the mechanism of action of DNA replication, recombination, and repair. Furthermore, methods for regulating or altering the cell cycle is needed.

Related Literature

Braun *et al.* (1997) *Biochemistry* 36:8443-8454; report on the role of protein-protein interactions and the function of replication protein A. It is reported that RPA modulates the activity of DNA polymerase α by multiple mechanisms.

Loor *et al.* (1997) *Nucleic Acids Research* 25:5041-5046 report on the identification of DNA replication in cell cycle proteins that interact with proliferating cell nuclear antigen.

Longhese *et al.* (1994) *Molecular and Cellular Biology* 14:7884-7890 report that replication factor A is required for *in vivo* DNA replication, repair, and recombination.

Stigger *et al.* (1998) *J. Biol. Chem.* 273:9337-9343 provide a functional analysis of human replication protein A in nucleotide excision repair.

Abremova *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:7186-7191 report that the interaction between replication protein A and p53 is disrupted after ultraviolet damage in a DNA repair-dependent manner.

New *et al.* (1998) *Nature* 391:407-410 reports that RAD52 protein stimulates DNA strand exchange by RAD51 and replication protein A. Stimulation was dependent on the concerted action of both RAD51 protein and RPA implying that specific protein-protein interactions between RAD52 protein, RAD51 protein and RPA are required.

Dutta *et al.* (1992) *EMBO J* 11(6):2189-2199 and Niu *et al.* (1997) *J. Biol. Chem.* 272(19):12634-41 report cell cycle-dependent phosphorylation of the middle subunit of RPA, implying a role for the subunit in cell cycle regulation.

Bochkareva *et al.* (1998) *J. Biol. Chem.* 273(7):3932-3936 report the
5 formation of a single stranded DNA binding site on the human RPA middle subunit.

Mass *et al.* (1998) *Mol. Cell. Biol.* 18(11):6399-6407 report that the RPA middle subunit contacts nascent simian virus 40 DNA, particularly the early DNA chain intermediates synthesized by DNA polymerase alpha-primase (RNA-DNA
10 primers), but not more advanced products.

Lavrik *et al.* (1998) *Nucleic Acids Res* 26(2):602-607 report on location of binding of individual subunits of human RPA to DNA primer-template complexes in various elongation reactions.

Sibenaller *et al.* (1998) 37(36):12496-12506 report that differences in the
15 activity of the middle (32kDa) and the small (14 Kda) subunits of RPA are responsible for variations in the single stranded DNA-binding properties of *saccharomyces cerevisiae* and human RPA, thus implying a role for the subunits in species-specificity of DNA binding of RPA.

20 SUMMARY OF THE INVENTION

Compositions and methods for modulating DNA metabolism in a host cell is provided. Particularly, the complete cDNA and amino acid sequence for homologues of maize replication protein A (RPA) large- and middle subunits are provided. The sequences of the invention find use in modulating DNA replication,
25 DNA repair, and recombination.

Transformed plants can be obtained having altered metabolic states. The invention has implications in genetic transformation and gene targeting in plants. Additionally, the methods can be used to promote cell death particularly in an inducible or tissue-preferred manner.

30

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a comparison of eukaryotic RPA large subunit amino acid sequences. Amino acid sequences for the RPA large subunits *from*

- *Sacchromyces Cerevisiae* (Rfal_Yeast, SEQ ID NO:10), *Schizosacchromyces pombe* (Rfal_Schpo, SEQ ID NO: 9), *Drosophila melanogaster* (Rfal_Drome, SEQ ID NO:8), *Homo sapiens* (Rfal_Human, SEQ ID NO: 7), *Xenopus laevis* (Rfa_Xenla, SEQ ID NO: 6), and *Oryza sativa* (O24183, SEQ ID NO:5) were compared with the maize RPA LS homologue 1 (ZMRPALSH1, SEQ ID NO:2) and homologue 2 (ZMRPALSH2, SEQ ID NO:4) using the GCG PileUp program utilizing default parameters. The putative zinc finger region is shown in italics.

Figure 2 provides an expression construct for inducible expression of the maize RPA large or middle subunit antisense construct.

DETAILED DESCRIPTION OF THE INVENTION

Nucleotide sequences and proteins useful for modulating DNA metabolism are provided. The nucleotide and amino acid sequences correspond to the maize replication protein A (RPA) subunits. RPA is a single-stranded DNA-binding protein that is required for multiple processes in DNA metabolism, including DNA replication, DNA repair, and recombination. The RPA complex generally comprises subunits of approximately 70, 32, and 14 kDa. By "large subunit", "middle subunit", and "small subunit" is herein intended a RPA subunit having the approximate molecular weight of 70-, 32-, and 14 kDa respectively. The sequences of the invention comprise the large- and middle subunits of the RPA complex. The sequences of the invention additionally find use in modulating gene expression.

Compositions of the invention include RPA nucleotide and amino acid sequences that are involved in modulating DNA metabolism. In particular, the present invention provides for isolated nucleic acid molecules comprising nucleotide sequences encoding the amino acid sequences shown in SEQ ID NOs:2 and 4 for the large subunit, and SEQ ID NOs: 12, 14, 16, 18, 20, and 22 for the middle subunit. SEQ ID NO:2 and SEQ ID NO:4 correspond to the amino acid sequences for the maize RPA large subunit homologue 1 (ZmRPALSH1) and homologue 2 (ZmRPALSH2). SEQ ID NOs: 12, 14, 16, 18, 20, and 22 correspond to the amino acid sequences for the maize middle subunit homologue 1 (ZmRPAMSH1); homologues 2 and 3 (ZmRPAMSH2 and ZmRPAMSH3);

homologue 4 (ZmRPAMSH4); homologue 5 (ZmRPAMSH5); homologue 6 (ZmRPAMSH6); and homologue 7 (ZmRPAMSH7) respectively.

For the large subunit, the present invention alternatively provides the nucleotide sequences encoding the DNA sequences deposited in a bacterial host as Patent Deposit Nos: 98754 and 98843. For the large subunits, further are polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein, for example those set forth in SEQ ID NOs: 1 and 3, those deposited in a bacterial host as Patent Deposit Nos: 98754 and 98843, and fragments and variants thereof.

Plasmids containing the RPA large subunit nucleotide sequences of the invention were deposited with the Patent Depository of the American Type Culture Collection (ATCC), Manassas, Virginia, and assigned Patent Deposit NOs: 98754 and 98843. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

Nucleotide sequences encoding the amino acid sequences for the maize RPA large subunit homologue 1 (ZmRPALSH1) and homologue 2 (ZmRPALSH2) are set forth in SEQ ID NOs 1 and 3. Nucleotide sequences encoding the amino acid sequences for the maize RPA middle subunit homologue 1 (ZmRPAMSH1); homologues 2 and 3 (ZmRPAMSH2 and ZmRPAMSH3); homologue 4 (ZmRPAMSH4); homologue 5 (ZmRPAMSH5); homologue 6 (ZmRPAMSH6); and homologue 7 (ZmRPAMSH7) are set forth in SEQ ID NOs: 11, 13, 15, 17, 19, and 21 respectively.

The invention encompasses isolated or substantially purified nucleic acid or protein compositions. An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Preferably, an "isolated" nucleic acid is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from

which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5%, (by dry weight) of contaminating protein. When the protein of the invention or biologically active portion thereof is recombinantly produced, preferably culture medium represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

RPA binds tightly to single-stranded DNA (ssDNA). The affinity of binding to double-stranded DNA (dsDNA) is three to four orders of magnitude lower than the binding affinity for ssDNA. Because RPA has been found to bind specifically to certain dsDNA sequences that seem to be involved in the regulation of transcription, modulation of gene expression may be affected by an increase or decrease in RPA expression in the host cell.

RPA has a wide range of activity and therefore uses relating to DNA metabolism and cell cycle. RPA interacts specifically with several proteins required for nucleotide excision repair. Interactions with repair proteins indicate that RPA may be important for efficient damage recognition and cleavage. RPA additionally interacts with RAD52 protein, a protein that is essential for dsDNA-break repair. This interaction appears to be essential for homologous recombination. In this manner, expression of the nucleotides of the invention may promote homologous recombination by recruiting factors which are essential for recombination to occur. Thus, the methods and compositions of the invention find use in promoting homologous recombination.

In one embodiment, genetic manipulation by homologous recombination can be improved by either expression of the RPA coding sequences of the invention during transformation, or by providing RPA protein. RPA protein, for example, may be provided as a coating to particles during particle bombardment. Alternatively, DNA constructs providing for the expression of RPA may be included with the DNA to be transformed. The increase in RPA during transformation, particularly integration of polynucleotides by homologous

recombination, promotes integration and insertion of the DNA sequences of interest into the plant genome.

In the same manner, it may be beneficial to inhibit the expression or presence of the RPA protein to encourage non-specific recombination events. In this manner, antibodies, peptides, antisense oligonucleotides and the like may be utilized to inhibit the activity of RPA. Alternatively, antisense constructs may be provided to inhibit the expression of RPA and encourage non-specific recombination.

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of plant genes. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. The design and use of target RNA-specific ribozymes is described in Haseloff *et al.* (1988) *Nature* 334:585-591.

A variety of cross-linking agents, alkylating agents and radical generating species as pendant groups on polynucleotides of the present invention can be used to bind, label, detect, and/or cleave nucleic acids. For example, Vlassov, V. V. *et al.* (1986) *Nucleic Acids Res.* 14:4065-4076, describe covalent bonding of a single-stranded DNA fragment with alkylating derivatives of nucleotides complementary to target sequences. A report of similar work by the same group is that by Knorre *et al.* (1985) *Biochimie* 67:785-789. Iverson and Dervan also showed sequence-specific cleavage of single-stranded DNA mediated by incorporation of a modified nucleotide which was capable of activating cleavage (1987) *J. Am. Chem. Soc.* 109:1241-1243). Meyer *et al.* (1989) *J. Am. Chem. Soc.* 111:8517-8519, effect covalent crosslinking to a target nucleotide using an alkylating agent complementary to the single-stranded target nucleotide sequence. A photoactivated crosslinking to single-stranded oligonucleotides mediated by psoralen was disclosed by Lee *et al.* (1988) *Biochem.* 27:3197-3203. Use of crosslinking in triple-helix forming probes was also disclosed by Home *et al.*

- (1990) *J. Am. Chem. Soc.* 112:2435-2437. Use of N4, N4-ethanocytosine as an alkylating agent to crosslink to single-stranded oligonucleotides has also been described by Webb *et al.* (1986) *J. Am. Chem. Soc.* 108:2764-2765; Webb *et al.* (1986) *Nucleic Acids Res.* 14:7661-7674; Feteritz *et al.* (1991) *J. Am. Chem. Soc.* 113:4000. Various compounds to bind, detect, label, and/or cleave nucleic acids are known in the art. See, for example, U.S. Patent Nos. 5,543,507; 5,672,593; 5,484,908; 5,256,648; and 5,681,941.

RPA is required for the replication of chromosomal DNA. Inhibition of endogenous RPA expression is deleterious to the cell, organism, or plant. Thus, the constructs of the invention can be used to selectively kill target cells or tissues. This can be accomplished through the use of inducible or tissue-preferred promoters. In this manner, the sequences of the invention may find use in enhancing pathogen resistance. An antisense construct for the RPA coding sequence is operably linked to a pathogen-inducible promoter. Upon contact with the pathogen, the RPA antisense construct is expressed resulting in cell death and effectively preventing the invasion of the pathogen.

The invention is drawn to compositions and methods for inducing resistance in a plant to plant pests. Accordingly, the compositions and methods are also useful in protecting plants against fungal pathogens, viruses, nematodes, insects and the like.

By "disease resistance" is intended that the plants avoid the disease symptoms that are the outcome of plant-pathogen interactions. That is, pathogens are prevented from causing plant diseases and the associated disease symptoms, or alternatively, the disease symptoms caused by the pathogen is minimized or lessened. The methods of the invention can be utilized to protect plants from disease, particularly those diseases that are caused by plant pathogens.

Pathogens of the invention include, but are not limited to, viruses or viroids, bacteria, insects, nematodes, fungi, and the like. Viruses include any plant virus, for example, tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, maize dwarf mosaic virus, etc. Specific fungal and viral pathogens for the major crops include: Soybeans: *Phytophthora megasperma* fsp. *glycinea*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *sojae* (*Phomopsis sojae*), *Diaporthe*

- phaseolorum* var. *caulivora*, *Sclerotium rolfsii*, *Cercospora kikuchii*, *Cercospora*
sojina, *Peronospora manshurica*, *Colletotrichum dematium* (*Colletotrichum*
truncatum), *Corynespora cassicola*, *Septoria glycines*, *Phyllosticta sojicola*,
Alternaria alternata, *Pseudomonas syringae* p.v. *glycinea*, *Xanthomonas*
5 *campestris* p.v. *phaseoli*, *Microsphaera diffusa*, *Fusarium semitectum*,
Phialophora gregata, Soybean mosaic virus, *Glomerella glycines*, Tobacco Ring
spot virus, Tobacco Streak virus, *Phakopsora pachyrhizi*, *Pythium*
aphanidermatum, *Pythium ultimum*, *Pythium debaryanum*, Tomato spotted wilt
virus, *Heterodera glycines*, *Fusarium solani*; Canola: *Albugo candida*, *Alternaria*
10 *brassicae*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*,
Mycosphaerella brassicicola, *Pythium ultimum*, *Peronospora parasitica*, *Fusarium*
roseum, *Alternaria alternata*; Alfalfa: *Clavibacter michiganense* subsp. *insidiosum*,
Pythium ultimum, *Pythium irregulare*, *Pythium splendens*, *Pythium debaryanum*,
Pythium aphanidermatum, *Phytophthora megasperma*, *Peronospora trifoliorum*,
15 *Phoma medicaginis* var. *medicaginis*, *Cercospora medicaginis*, *Pseudopeziza*
medicaginis, *Leptotrochila medicaginis*, *Fusarium*, *Xanthomonas campestris* p.v.
alfalfae, *Aphanomyces euteiches*, *Stemphylium herbarum*, *Stemphylium alfalfae*;
Wheat: *Pseudomonas syringae* p.v. *atrofaciens*, *Urocystis agropyri*, *Xanthomonas*
campestris p.v. *translucens*, *Pseudomonas syringae* p.v. *syringae*, *Alternaria*
20 *alternata*, *Cladosporium herbarum*, *Fusarium graminearum*, *Fusarium*
avenaceum, *Fusarium culmorum*, *Ustilago tritici*, *Ascochyta tritici*,
Cephalosporium gramineum, *Colletotrichum graminicola*, *Erysiphe graminis* f.sp.
tritici, *Puccinia graminis* f.sp. *tritici*, *Puccinia recondita* f.sp. *tritici*, *Puccinia*
striiformis, *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Septoria tritici*,
25 *Septoria avenae*, *Pseudocercospora herpotrichoides*, *Rhizoctonia solani*,
Rhizoctonia cerealis, *Gaeumannomyces graminis* var. *tritici*, *Pythium*
aphanidermatum, *Pythium arrhenomanes*, *Pythium ultimum*, *Bipolaris*
sorokiniana, Barley Yellow Dwarf Virus, Brome Mosaic Virus, Soil Borne Wheat
Mosaic Virus, Wheat Streak Mosaic Virus, Wheat Spindle Streak Virus, American
30 Wheat Striate Virus, *Claviceps purpurea*, *Tilletia tritici*, *Tilletia laevis*, *Ustilago*
tritici, *Tilletia indica*, *Rhizoctonia solani*, *Pythium arrhenomanes*, *Pythium*
gramicola, *Pythium aphanidermatum*, High Plains Virus, European wheat striate
virus; Sunflower: *Plasmophora halstedii*, *Sclerotinia sclerotiorum*, Aster Yellows,

- *Septoria helianthi*, *Phomopsis helianthi*, *Alternaria helianthi*, *Alternaria zinniae*,
Botrytis cinerea, *Phoma macdonaldii*, *Macrophomina phaseolina*, *Erysiphe*
cichoracearum, *Rhizopus oryzae*, *Rhizopus arrhizus*, *Rhizopus stolonifer*, *Puccinia*
helianthi, *Verticillium dahliae*, *Erwinia carotovorum* pv. *carotovora*,
- 5 *Cephalosporium acremonium*, *Phytophthora cryptogea*, *Albugo tragopogonis*;
Corn: *Fusarium moniliforme* var. *subglutinans*, *Erwinia stewartii*, *Fusarium*
moniliforme, *Gibberella zeae* (*Fusarium graminearum*), *Stenocarpella maydi*
(*Diplodia maydis*), *Pythium irregulare*, *Pythium debaryanum*, *Pythium*
graminicola, *Pythium splendens*, *Pythium ultimum*, *Pythium aphanidermatum*,
- 10 *Aspergillus flavus*, *Bipolaris maydis* O, T (*Cochliobolus heterostrophus*),
Helminthosporium carbonum I, II & III (*Cochliobolus carbonum*), *Exserohilum*
turcicum I, II & III, *Helminthosporium pedicellatum*, *Physoderma maydis*,
Phyllosticta maydis, *Kabatiella maydis*, *Cercospora sorghi*, *Ustilago maydis*,
Puccinia sorghi, *Puccinia polysora*, *Macrophomina phaseolina*, *Penicillium*
- 15 *oxalicum*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Curvularia lunata*,
Curvularia inaequalis, *Curvularia pallescens*, *Clavibacter michiganense* subsp.
nebraskense, *Trichoderma viride*, Maize Dwarf Mosaic Virus A & B, Wheat
Streak Mosaic Virus, Maize Chlorotic Dwarf Virus, *Claviceps sorghi*,
Pseudomonas avenae, *Erwinia chrysanthemi* pv. *zea*, *Erwinia carotovora*, Corn
- 20 *stunt spiropasma*, *Diplodia macrospora*, *Sclerophthora macrospora*,
Peronosclerospora sorghi, *Peronosclerospora philippinensis*, *Peronosclerospora*
maydis, *Peronosclerospora sacchari*, *Sphacelotheca reiliana*, *Physopella zeae*,
Cephalosporium maydis, *Cephalosporium acremonium*, Maize Chlorotic Mottle
Virus, High Plains Virus, Maize Mosaic Virus, Maize Rayado Fino Virus, Maize
- 25 Streak Virus, Maize Stripe Virus, Maize Rough Dwarf Virus; Sorghum:
Exserohilum turcicum, *Colletotrichum graminicola* (*Glomerella graminicola*),
Cercospora sorghi, *Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas*
syringae p.v. *syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas*
andropogonis, *Puccinia purpurea*, *Macrophomina phaseolina*, *Perconia circinata*,
- 30 *Fusarium moniliforme*, *Alternaria alternata*, *Bipolaris sorghicola*,
Helminthosporium sorghicola, *Curvularia lunata*, *Phoma insidiosa*, *Pseudomonas*
avenae (*Pseudomonas alboprecipitans*), *Ramulispora sorghi*, *Ramulispora*
sorghicola, *Phyllachara sacchari*, *Sporisorium reilianum* (*Sphacelotheca reiliana*),

Sphacelotheca cruenta, *Sporisorium sorghi*, Sugarcane mosaic H, Maize Dwarf Mosaic Virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophithona macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium graminicola*, etc.

Nematodes include parasitic nematodes such as root-knot, cyst, and lesion nematodes, including *Heterodera* and *Globodera* spp; particularly *Globodera rostochiensis* and *globodera pailida* (potato cyst nematodes); *Heterodera glycines* (soybean cyst nematode); *Heterodera schachtii* (beet cyst nematode); and

10 *Heterodera avenae* (cereal cyst nematode).

Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Coleoptera and Lepidoptera. Insect pests of the invention for the

15 major crops include: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, sugarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis barberi*,

20 northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus* spp., wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid;

25 *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscurus*, grass thrips; *Solenopsis milesta*, thief ant; *Tetranychus urticae*, twospotted spider mite; Sorghum: *Chilo partellus*,

30 sorghum borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes*, *Conoderus*, and *Aeolus* spp., wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema*

- *pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Sipha flava*, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite;
- 5 Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, western cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata howardi*, southern corn rootworm; Russian wheat aphid;
- 10 *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella*
- 15 *fusca*, tobacco thrips; *Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton
- 20 bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus grandis grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential
- 25 grasshopper; *Thrips tabaci*, onion thrips; *Franklinkiella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil;
- 30 *Nephotettix nigropictus*, rice leafhopper; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; Soybean: *Pseudopiusia includens*, soybean looper; *Anticarsia gemmatilis*, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black

cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestanii*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* spp., Root maggots.

A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. The nucleic acids can be combined with constitutive, tissue-preferred, or other promoters for expression in plants.

A plant promoter can be employed which will direct expression of a polynucleotide of the present invention in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Such constitutive promoters include, for example, the core promoter of the Rsyn7 (WO 99/43838); the core CaMV 35S promoter (Odell *et al.* (1985) *Nature* 313:810-812); rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten *et al.* (1984) *EMBO J.* 3:2723-2730); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142.

Alternatively, the plant promoter can direct expression of a polynucleotide of present invention in a specific tissue or may be otherwise under more precise

- environmental or developmental control. Such promoters are referred to here as "inducible" promoters. Environmental conditions that may effect transcription by inducible promoters include pathogen attack, anaerobic conditions, or the presence of light. Examples of inducible promoters are the Adhl promoter which is
- 5 inducible by hypoxia or cold stress, the Hsp70 promoter which is inducible by heat stress, and the PPKK promoter which is inducible by light.

Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. An exemplary promoter is the anther specific promoter

10 5126 (U.S. Patent Nos. 5,689,049 and 5,689,051). The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

The promoters can be selected based on the desired outcome. When the genes are expressed at levels to cause cell death, an inducible promoter or tissue

15 specific promoters can be used to drive the expression of the genes of the invention. The inducible promoter must be tightly regulated to prevent unnecessary cell death, yet be expressed in the presence of a pathogen to prevent infection and disease symptoms.

Generally, it will be beneficial to express the gene from an inducible promoter,

20 particularly from a pathogen-inducible promoter. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi *et al.* (1983) *Neth. J. Plant Pathol.* 89:245-254; Uknes *et al.* (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant*

25 *Mol. Virol.* 4:111-116. See also the copending application entitled "Inducible Maize Promoters"; U.S. Application Serial No. 09/257,583, filed February 25, 1999, herein incorporated by reference.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau *et al.* (1987) *Plant Mol. Biol.*

30 9:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions* 2:325-331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch *et al.* (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, Chen *et al.* (1996) *Plant J.* 10:955-966; Zhang *et al.*

(1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner *et al.* (1993) *Plant J.* 3:191-201; Siebertz *et al.* (1989) *Plant Cell* 1:961-968; U.S. Patent No. 5,750,386 (nematode-inducible); and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero *et al.* (1992) *Physiol. Mol. Plant Path.* 41:189-200).

Additionally, as pathogens find entry into plants through wounds or insect damage, a wound-inducible promoter may be used in the constructions of the invention. Such wound-inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan (1990) *Ann. Rev. Phytopath.* 28:425-449; Duan *et al.* (1996) *Nature Biotechnology* 14:494-498); *wun1* and *wun2*, US Patent No. 5,428,148; *win1* and *win2* (Stanford *et al.* (1989) *Mol. Gen. Genet.* 215:200-208); systemin (McGurl *et al.* (1992) *Science* 225:1570-1573); WIP1 (Rohmeier *et al.* (1993) *Plant Mol. Biol.* 22:783-792; Eckelkamp *et al.* (1993) *FEBS Letters* 323:73-76); MPI gene (Corderok *et al.* (1994) *Plant J.* 6(2):141-150); and the like, herein incorporated by reference.

Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of the chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize In2-2 promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-1a promoter, which is activated by salicylic acid. Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:10421-10425 and McNellis *et al.* (1998) *Plant J.* 14(2):247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz *et al.* (1991) *Mol. Gen. Genet.* 227:229-237, and U.S. Patent Nos. 5,814,618 and 5,789,156), herein incorporated by reference.

Where low level expression is desired, weak promoters will be used.

Generally, by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By low level is intended at levels of about 1/1000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts.

5 Alternatively, it is recognized that weak promoters also encompasses promoters that are expressed in only a few cells and not in others to give a total low level of expression. Where a promoter is expressed at unacceptably high levels, portions of the promoter sequence can be deleted or modified to decrease expression levels.

Such weak constitutive promoters include, for example, the core promoter
10 of the Rsyn7 (WO 99/43838), the core 35S CaMV promoter, and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142. See also, the copending application entitled "Constitutive Maize Promoters", U.S. Application Serial No. 09/257,584, filed February 25, 1999, and herein
15 incorporated by reference.

Tissue-preferred promoters can be utilized to target enhanced RPA expression within a particular plant tissue. In this aspect of the invention, the antisense constructs are useful for tissue-preferred expression. Male or female sterility may be affected by use of the antisense constructs with tissue-preferred
20 promoters. Although not a limitation, of particular interest are promoters for male sterility. For example, the anther-preferred promoter 5126 can be used. See, for example, U.S. Patent Nos. 5,689,049 and 5,689,051, herein incorporated by reference.

Tissue-preferred promoters include Yamamoto *et al.* (1997) *Plant J.*
25 12(2):255-265; Kawamata *et al.* (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen *et al.* (1997) *Mol. Gen Genet.* 254(3):337-343; Russell *et al.* (1997) *Transgenic Res.* 6(2):157-168; Rinehart *et al.* (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp *et al.* (1996) *Plant Physiol.* 112(2):525-535; Canevascini *et al.* (1996) *Plant Physiol.* 112(2):513-524; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-
30 778; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; Orozco *et al.* (1993) *Plant Mol Biol.* 23(6):1129-1138; Matsuoka *et al.* (1993) *Proc Natl. Acad. Sci. USA* 90(20):9586-9590; and Guevara-Garcia *et al.* (1993) *Plant J.* 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

Leaf-specific promoters are known in the art. See, for example, Yamamoto *et al.* (1997) *Plant J.* 12(2):255-265; Kwon *et al.* (1994) *Plant Physiol.* 105:357-67; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-778; Gotor *et al.* (1993) *Plant J.* 3:509-18; Orozco *et al.* (1993) *Plant Mol. Biol.* 23(6):1129-1138; and

5 Matsuoka *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90(20):9586-9590.

Root-specific promoters are known and can be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire *et al.* (1992) *Plant Mol. Biol.* 20(2): 207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner (1991) *Plant Cell*

10 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger *et al.* (1990) *Plant Mol. Biol.* 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao *et al.* (1991) *Plant Cell* 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of

15 soybean). See also Bogusz *et al.* (1990) *Plant Cell* 2(7):633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are described. The promoters of these genes were linked to a β -glucuronidase reporter gene and introduced into both the nonlegume *Nicotiana*

20 *tabacum* and the legume *Lotus corniculatus*, and in both instances root-specific promoter activity was preserved. Leach and Aoyagi (1991) describe their analysis of the promoters of the highly expressed rolC and rolD root-inducing genes of *Agrobacterium rhizogenes* (see *Plant Science* (Limerick) 79(1):69-76). They concluded that enhancer and tissue-preferred DNA determinants are dissociated in

25 those promoters. Teeri *et al.* (1989) used gene fusion to lacZ to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see *EMBO J.*

30 8(2):343-350). The TR1' gene, fused to *nptII* (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VtENOD-GRP3 gene promoter (Kuster *et al.* (1995) *Plant Mol. Biol.* 29(4):759-772); and rolB promoter (Capana *et al.* (1994) *Plant Mol. Biol.* 25(4):681-691. See

- also U.S. Patent Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

“Seed-preferred” promoters include both “seed-specific” promoters (those promoters active during seed development such as promoters of seed storage proteins) as well as “seed-germinating” promoters (those promoters active during seed germination). See Thompson *et al.* (1989) *BioEssays* 10:108, herein incorporated by reference. Such seed-preferred promoters include, but are not limited to, Cim1 (cytokinin-induced message); cZ19B1 (maize 19 kDa zein); milps (myo-inositol-1-phosphate synthase); and celA (cellulose synthase) (see the copending application entitled “Seed-Preferred Promoters,” U.S. Application Serial No. 60/097,233, filed August 20, 1998, herein incorporated by reference. Gama-zein is a preferred endosperm-specific promoter. Glob-1 is a preferred embryo-specific promoter. For dicots, seed-specific promoters include, but are not limited to, bean β -phaseolin, napin, β -conglycinin, soybean lectin, cruciferin, and the like. For monocots, seed-specific promoters include, but are not limited to, maize 15 kDa zein, 22 kDa zein, 27 kDa zein, g-zein, waxy, shrunken 1, shrunken 2, globulin 1, etc.

Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention. These promoters can also be used, for example, in recombinant expression cassettes to drive expression of antisense nucleic acids to reduce, increase, or alter RPA content and/or composition in a desired tissue, or to generate sterile plants. Optionally, RPA nucleic acids from a variety of sources, as discussed above can be employed to create male sterile plants. In optional embodiments, the RPA gene or cDNA is operably linked to an anther-specific promoter such as 5126, as discussed above. Preferably, the male sterile plant is maize.

Thus, in some embodiments, the nucleic acid construct will comprise a promoter functional in a plant cell, such as in *Zea mays*, operably linked to a polynucleotide of the present invention. Promoters useful in these embodiments include the endogenous promoters driving expression of a polypeptide of the present invention.

In some embodiments, isolated nucleic acids which serve as promoter or enhancer elements can be introduced in the appropriate position (generally

upstream) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* by mutation, deletion, and/or substitution (see, Kmiec, U.S. Patent No. 5,565,350; Zarling *et al.*,
5 PCT/US93/03868), or isolated promoters can be introduced into a plant cell in the proper orientation and distance from a RPA gene so as to control the expression of the gene. Gene expression can be modulated under conditions suitable for plant growth so as to alter RPA content and/or composition. Thus, the present invention provides compositions, and methods for making, heterologous promoters and/or
10 enhancers operably linked to a native, endogenous (i.e., non-heterologous) form of a polynucleotide of the present invention.

Methods for identifying promoters with a particular expression pattern, in terms of e.g., tissue type, cell type, stage of development, and/or environmental conditions, are well known in the art. See, e.g., *The Maize Handbook*, Chapters
15 114-115, Freeling and Walbot, eds., Springer, New York (1994); *Corn and Corn Improvement*, 3rd edition, Chapter 6, Sprague and Dudley, eds., American Society of Agronomy, Madison, Wisconsin (1988). A typical step in promoter isolation methods is identification of gene products that are expressed with some degree of specificity in the target tissue. Amongst the range of methodologies are:
20 differential hybridization to cDNA libraries; subtractive hybridization; differential display; differential 2-D protein gel electrophoresis; DNA probe arrays; and isolation of proteins known to be expressed with some specificity in the target tissue. Such methods are well known to those of skill in the art. Commercially available products for identifying promoters are known in the art such as
25 Clontech's (Palo Alto, CA) Universal GenomeWalker Kit.

For the protein-based methods, it is helpful to obtain the amino acid sequence for at least a portion of the identified protein, and then to use the protein sequence as the basis for preparing a nucleic acid that can be used as a probe to identify either genomic DNA directly, or preferably, to identify a cDNA clone
30 from a library prepared from the target tissue. Once such a cDNA clone has been identified, that sequence can be used to identify the sequence at the 5' end of the transcript of the indicated gene. For differential hybridization, subtractive hybridization and differential display, the nucleic acid sequence identified as

- enriched in the target tissue is used to identify the sequence at the 5' end of the transcript of the indicated gene. Once such sequences are identified, starting either from protein sequences or nucleic acid sequences, any of these sequences identified as being from the gene transcript can be used to screen a genomic library prepared from the target organism. Methods for identifying and confirming the transcriptional start site are well known in the art.

In the process of isolating promoters expressed under particular environmental conditions or stresses, or in specific tissues, or at particular developmental stages, a number of genes are identified that are expressed under the desired circumstances, in the desired tissue, or at the desired stage. Further analysis will reveal expression of each particular gene in one or more other tissues of the plant. One can identify a promoter with activity in the desired tissue or condition but that do not have activity in any other common tissue.

To identify the promoter sequence, the 5' portions of the clones described here are analyzed for sequences characteristic of promoter sequences. For instance, promoter sequence elements include the TATA box consensus sequence (TATAAT), which is usually an AT-rich stretch of 5-10 bp located approximately 20 to 40 base pairs upstream of the transcription start site. Identification of the TATA box is well known in the art. For example, one way to predict the location of this element is to identify the transcription start site using standard RNA-mapping techniques such as primer extension, S1 analysis, and/or RNase protection. To confirm the presence of the AT-rich sequence, a structure-function analysis can be performed involving mutagenesis of the putative region and quantification of the mutation's effect on expression of a linked downstream reporter gene. See, e.g., *The Maize Handbook*, Chapter 114, Freeling and Walbot, eds., Springer, New York (1994).

In plants, further upstream from the TATA box, at positions -80 to -100, there is typically a promoter element (i.e., the CAAT box) with a series of adenines surrounding the trinucleotide G (or T) N G. J. Messing *et al.*, in *Genetic Engineering in Plants*, Kosage, Meredith and Hollaender, eds., pp. 221-227 (1983). In maize, there no well-conserved CAAT box but there are several short, conserved protein-binding motifs upstream of the TATA box. These include motifs for the transacting transcription factors involved in light regulation,

anaerobic induction, hormonal regulation, or anthocyanin biosynthesis, as appropriate for each gene.

Once promoter and/or gene sequences are known, a region of suitable size is selected from the genomic DNA that is 5' to the transcriptional start, or the translational start site, and such sequences are then linked to a coding sequence. If the transcriptional start site is used as the point of fusion, any of a number of possible 5' untranslated regions can be used in between the transcriptional start site and the partial coding sequence. If the translational start site at the 3' end of the specific promoter is used, then it is linked directly to the methionine start codon of a coding sequence.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added can be derived from, example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

An intron sequence can be added to the 5' untranslated region or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold. Buchman *et al.* (1988) *Mol. Cell Biol.* 8:4395-4405; Callis *et al.* (1987) *Genes Dev.* 1:1183-1200. Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of maize introns Adhl-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. See generally, *The Maize Handbook*, Chapter 116, Freeling and Walbot, eds., Springer, New York (1994).

The vector comprising the sequences from a polynucleotide of the present invention could comprise a selectable marker gene for the selection of transformed cells or tissues. Selectable marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal

- compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). See generally, Yarranton (1992) *Curr. Opin. Biotech.* 3:506-511; Christopherson *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6314-6318; Yao *et al.* (1992) *Cell* 71:63-72; Reznikoff (1992) *Mol. Microbiol.* 6:2419-2422; Barkley *et al.* (1980) in *The Operon*, pp. 177-220; Hu *et al.* (1987) *Cell* 48:555-566; Brown *et al.* (1987) *Cell* 49:603-612; Figge *et al.* (1988) *Cell* 52:713-722; Deuschle *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:5400-5404; Fuerst *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-2553; Deuschle *et al.* (1990) *Science* 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-1921; Labow *et al.* (1990) *Mol. Cell. Biol.* 10:3343-3356; Zambretti *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-3956; Baim *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-5076; Wyborski *et al.* (1991) *Nucleic Acids Res.* 19:4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10:143-162; Degenkolb *et al.* (1991) *Antimicrob. Agents Chemother.* 35:1591-1595; Kleinschmidt *et al.* (1988) *Biochemistry* 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Oliva *et al.* (1992) *Antimicrob. Agents Chemother.* 36:913-919; Hlavka *et al.* (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill *et al.* (1988) *Nature* 334:721-724. Such disclosures are herein incorporated by reference.

The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

- Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers *et al.* (1987) *Meth. in Enzymol.* 153:253-277. These vectors are plant integrating vectors in that on transformation, the vectors integrate a portion of vector DNA into the genome of the host plant. Exemplary *A. tumefaciens* vectors useful herein are plasmids pKYLX6 and pKYLX7 of Schardl *et al.* (1987) *Gene* 61:1-11 and Berger *et al.* (1989) *Proc. Natl. Acad. Sci. (USA)* 86:8402-8406. Another useful vector herein is plasmid pBI101.2 that is available from Clontech Laboratories, Inc. (Palo Alto, CA).

As discussed above, a polynucleotide of the present invention can be expressed in either sense or antisense orientation as desired. It will be appreciated

that control of gene expression in either sense or antisense orientation can have a direct impact on the observable plant characteristics. Antisense technology can be conveniently used for gene expression in plants. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such
5 that the antisense strand of RNA will be transcribed. The construct is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been shown that antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, see, e.g., Sheehy *et al.* (1988) *Proc. Natl. Acad. Sci. (USA)* 85:8805-8809; and Hiatt *et al.*,
10 U.S. Patent No. 4,801,340.

In the methods of the invention, it is recognized that the entire coding sequence for the RPA construct may be utilized. Alternatively, portions or fragments of the sequence may be used in DNA constructs.

Fragments and variants of the disclosed nucleotide sequences and proteins
15 encoded thereby are encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein and hence modulate DNA metabolism. Alternatively, fragments of a
20 nucleotide sequence that are useful as hybridization probes generally do not encode fragment proteins retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length nucleotide sequence encoding the proteins of the invention.

25 A fragment of a RPA nucleotide sequence that encodes a biologically active portion of a RPA protein of the invention will encode at least 15, 25, 30, 50, 100, 150, 200, or 250 contiguous amino acids, or up to the total number of amino acids present in a full-length RPA protein of the invention (for example, 623, 617, 273, 273, 273, 318, 273, 273 amino acids for SEQ ID NOs: 2, 4, 12, 14, 16, 18,
30 20, and 22 respectively. Fragments of a RPA nucleotide sequence that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of a RPA protein.

- Thus, a fragment of a RPA nucleotide sequence may encode a biologically active portion of a RPA protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. A biologically active portion of a RPA protein can be prepared by isolating a portion of one of the RPA nucleotide sequences of the invention, expressing the encoded portion of the RPA protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the RPA protein. Nucleic acid molecules that are fragments of a RPA nucleotide sequence comprise at least 16, 20, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1,000 nucleotides, or up to the number of nucleotides present in a full-length RPA nucleotide sequence disclosed herein (for example, 2497, 2202, 1124, 979, 1051, 1087, 1074, and 1231 nucleotides for SEQ ID NOs: 1, 3, 11, 13, 15, 17, 19, and 21 respectively).

By "variants" is intended substantially similar sequences. For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the RPA polypeptides of the invention. Such naturally occurring variants including naturally occurring allelic variants, can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a RPA protein of the invention. Generally, variants of a particular nucleotide sequence of the invention will have at least 40%, 50%, 60%, 70%, generally at least 75%, 80%, 85%, preferably about 90% to 95% or more, and more preferably about 98% or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

By "variant" protein is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins

encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, modulating DNA metabolism as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a native RPA protein of the invention will have at least 40%, 50%, 60%, 70%,
5 generally at least 75%, 80%, 85%, preferably about 90% to 95% or more, and more preferably about 98% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs described elsewhere herein using default parameters. A biologically active variant of a protein of the invention may differ from that protein by as few as 1-15 amino acid
10 residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such
15 manipulations are generally known in the art. For example, amino acid sequence variants of the RPA proteins can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods in Enzymol.* 154:367-382; US Patent No. 4,873,192; Walker and Gastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing
20 Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein
25 incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred.

Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and
30 modified forms thereof. Such variants will continue to possess the desired activity in influencing DNA metabolism. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and

- preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444.

The deletions, insertions, and substitutions of the protein sequence encompassed herein are not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. That is, the activity can be evaluated by assessing DNA binding, recombination, repair and replication. See, for example, Braun *et al.* (1997) *Biochemistry* 36:8443-8454; Longhese *et al.* (1994) *Molecular and Cellular Biology* 14:7884-7890; Stigger *et al.* (1998) *J. Biol. Chem.* 273:9337-9343; Abremova *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:7186-7191; New *et al.* (1998) *Nature* 391:407-410; Bochkareva *et al.* (1998) *J. Biol. Chem.* 273(7):3932-6; Mass *et al.* (1998) *Mol. Cell. Biol.* 18(11):6399-407; Lavrik *et al.* (1998) *Nucleic Acids Res* 26(2):602-7; Sibenaller *et al.* (1998) 37(36):12496-506; Matsunaga *et al.* (1996) *J. Biol. Chem.* 271 (19): 11047-50; and Sung (1997) *Genes & Development* 11: 1111-21, herein incorporated by reference.

Variant nucleotide sequences and proteins also encompass nucleotide sequences and proteins derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different RPA coding sequences can be manipulated to create a new RPA possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between the RPA gene of the invention and other known RPA genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased K_m in the case of an enzyme. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751; Stemmer (1994) *Nature* 370:389-391; Cramer *et al.* (1997) *Nature Biotech.* 15:436-438; Moore *et al.* (1997) *J. Mol. Biol.* 272:336-347; Zhang *et al.* (1997) *Proc. Natl.*

Acad. Sci. USA 94:4504-4509; Cramer *et al.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

It is recognized that with these nucleotide sequences, antisense constructions, complementary to at least a portion of the messenger RNA (mRNA) for the RPA sequences can be constructed. Antisense nucleotides are constructed to hybridize with the corresponding mRNA. Modifications of the antisense sequences may be made as long as the sequences hybridize to and interfere with expression of the corresponding mRNA. In this manner, antisense constructions having 70%, preferably 80%, more preferably 85% sequence similarity to the corresponding antisense sequences may be used. Furthermore, portions of the antisense nucleotides may be used to disrupt the expression of the target gene. Generally, sequences of at least 50 nucleotides, 100 nucleotides, 200 nucleotides, or greater may be used.

The nucleotide sequences of the present invention may also be used in the sense orientation to suppress the expression of endogenous genes in plants. Methods for suppressing gene expression in plants using nucleotide sequences in the sense orientation are known in the art. The methods generally involve transforming plants with a DNA construct comprising a promoter that drives expression in a plant operably linked to at least a portion of a nucleotide sequence that corresponds to the transcript of the endogenous gene. Typically, such a nucleotide sequence has substantial sequence identity to the sequence of the transcript of the endogenous gene, preferably greater than about 65% sequence identity, more preferably greater than about 85% sequence identity, most preferably greater than about 95% sequence identity. See, U.S. Patent Nos. 5,283,184 and 5,034,323; herein incorporated by reference.

Use of the polypeptides and proteins, and fragments and variants thereof, for producing antibodies are also encompassed by the invention. The invention also encompasses using such antibodies to determine RPA protein levels, and to modulate one or more biological activities or interactions of RPA. Methods for the production of antibodies are known in the art. See, for example, Harlow and Lane, antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York (1988); and the reference is cited therein.

- The RPA sequences of the invention may be optimized for enhanced expression in plants of interest. See, for example, EPA0359472; WO91/16432; Perlak *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:3324-3328; and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498. In this manner, the genes can be synthesized utilizing plant-preferred condons. See, for example, Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, the disclosure of which is incorporated herein by reference. In this manner, synthetic genes can also be made based on the distribution of codons a particular host uses for a particular amino acid. Thus, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used.

Thus nucleotide sequences of the invention and the proteins encoded thereby include the native forms as well as variants thereof. The variant proteins will be substantially homologous and functionally equivalent to the native proteins. A variant of a native protein is "substantially homologous" to the native protein when at least about 80%, more preferably at least about 90%, and most preferably at least about 95% of its amino acid sequence is identical to the amino acid sequence of the native protein. By "functionally equivalent" is intended that the sequence of the variant defines a chain that produces a protein having substantially the same biological effect as the native protein of interest. Such functionally equivalent variants that comprise substantial sequence variations are also encompassed by the invention.

The nucleotide sequences of the invention can be used to isolate corresponding sequences from other organisms, particularly other plants, more particularly other monocots. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequence set forth herein. Sequences isolated based on their sequence identity to the entire RPA sequences set forth herein or to fragments thereof are encompassed by the present invention.

In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any plant of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook *et al.* (1989)

Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis *et al.*, eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P , or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the RPA sequences of the invention. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

For example, the entire RPA sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding RPA sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among RPA sequences and are preferably at least about 10 nucleotides in length, and most preferably at least about 20 nucleotides in length. Such probes may be used to amplify corresponding RPA sequences from a chosen plant by PCR. This technique may be used to isolate additional coding sequences from a desired plant or as a diagnostic assay to determine the presence of coding sequences in a plant. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook *et al.*

- (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the

length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Thus, isolated sequences that have promoter activity or encode for a RPA protein and which hybridize under stringent conditions to the RPA sequences disclosed herein, or to fragments thereof, are encompassed by the present invention. Such sequences will be at least 40% to 50% homologous, about 60% to 70% homologous, and even about 75%, 80%, 85%, 90%, 95% to 98% homologous or more with the disclosed sequences. That is, the sequence identity of sequences may range, sharing at least 40% to 50%, about 60% to 70%, and even about 75%, 80%, 85%, 90%, 95% to 98% or more sequence identity.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial identity".

5 (a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a
10 contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally
15 can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the
20 art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Preferred, non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; the homology alignment algorithm of Needleman and Wunsch (1970)
25 *J. Mol. Biol.* 48:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be
30 utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the

Wisconsin Genetics Software Package, Version 8 (available from Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al.* (1988) *Gene* 73:237-244 (1988); Higgins *et al.* (1989) *CABIOS* 5:151-153; Corpet *et al.* (1988) *Nucleic Acids Res.* 16:10881-90; Huang *et al.* (1992) *CABIOS* 8:155-65; and Pearson *et al.* (1994) *Meth. Mol. Biol.* 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See <http://www.ncbi.nlm.nih.gov>. Alignment may also be performed manually by inspection. Alignment may also be performed manually by inspection.

For purposes of the present invention, comparison of nucleotide or protein sequences for determination of percent sequence identity to the RPA sequences disclosed herein is preferably made using the GCG PileUp program, version 10.00, with its default parameters or any equivalent program. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by the preferred program.

- (c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

20 (d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

(e)(i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90%, and most preferably at least

95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. However, stringent conditions encompass temperatures in the range of about 1°C to about 20°C, depending upon the desired degree of stringency as otherwise qualified herein. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is when the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e)(ii) The term "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with at least 70% sequence identity to a reference sequence, preferably 80%, more preferably 85%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman *et al.* (1970) *J. Mol. Biol.* 48:443. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides that are "substantially similar" share sequences as noted above except that residue positions that are not identical may differ by conservative amino acid changes.

Using the nucleic acids of the present invention, one may express a protein of the present invention in a recombinantly engineered cell such as bacteria, yeast, insect, mammalian, or preferably plant cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location, and/or time), because they have been genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator. One of skill would recognize that modifications can be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located restriction sites or termination codons or purification sequences.

Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of *E. coli*; however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with

an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang *et al.* (1977) *Nature* 198:1056), the tryptophan (trp) promoter system (Goeddel *et al.* (1980) *Nucleic Acids Res.* 8:4057) and the lambda-derived P L promoter and N-gene ribosome binding site (Shimatake *et al.* (1981) *Nature* 292:128). The inclusion of selection markers in DNA vectors transfected in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol.

The vector is selected to allow introduction into the appropriate host cell.

10 Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein of the present invention are available using *Bacillus sp.* and *Salmonella* (Palva *et al.* (1983) *Gene* 15 22:229-235, Mosbach *et al.* (1983) *Nature* 302:543-545).

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. The sequences of the present invention can be expressed in these eukaryotic systems. In some embodiments, transformed/transfected plant cells are employed as expression

20 systems for production of the proteins of the instant invention.

Synthesis of heterologous proteins in yeast is well known. Sherman, F. *et al.* (1982) *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory is a well recognized work describing the various methods available to produce the protein in yeast. Two widely utilized yeast for production of eukaryotic proteins are

25 *Saccharomyces cerevisia* and *Pichia pastoris*. Vectors, strains, and protocols for expression in *Saccharomyces* and *Pichia* are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as promoters, including 3-phosphoglycerate kinase or alcohol oxidase, and an origin of replication, termination sequences and the like as

30 desired.

A protein of the present invention, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates. The monitoring of the purification process can be accomplished by using

Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

The sequences encoding proteins of the present invention can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect, or plant origin. Illustrative of cell cultures useful for the production of the peptides are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21, and CHO cell lines. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter (e.g., the CMV promoter, a HSV *tk* promoter or *pgk* (phosphoglycerate kinase promoter)), an enhancer (Queen *et al.* (1986) *Immunol. Rev.* 89:49), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. Other animal cells useful for production of proteins of the present invention are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th edition, 1992).

Appropriate vectors for expressing proteins of the present invention in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito larvae, silkworm, armyworm, moth and *Drosophila* cell lines such as a Schneider cell line (See Schneider *et al.* (1987) *J. Embryol. Exp. Morphol.* 27: 353-365).

As with yeast, when higher animal or plant host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague *et al.* (1983) *J. Virol.* 45:773-781). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus-type vectors. Saveria-Campo, M., Bovine Papilloma Virus DNA a Eukaryotic Cloning Vector in *DNA*

Cloning Vol. II a Practical Approach, D.M. Glover, ed., IRL Press, Arlington, Virginia pp. 213-238 (1985).

The sequences of the invention can be introduced into any plant of interest, and used for transformation of any plant species. The sequences to be introduced
5 may be used in expression cassettes for expression in the particular plant of interest.

Plants of interest include, but are not limited to corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale
10 cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*),
15 cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Cofea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*),
20 papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables, ornamentals, and conifers.

Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*),
25 peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia
30 (*Euphorbia pulcherrima*), and chrysanthemum. Conifers that may be employed in practicing the present invention include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir

(*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). Preferably, plants of the present invention are crop plants (for example, corn, alfalfa, sunflower, *Brassica*, soybean, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, etc.), more preferably corn and soybean plants, yet more preferably corn plants.

Plants of particular interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

The RPA coding and antisense sequences of the invention are provided in expression cassettes for expression in the plant of interest. The cassette will include 5' and 3' regulatory sequences operably linked to a RPA sequence of the invention. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on another expression cassette. By "operably linked" is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the RPA sequence to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a RPA DNA sequence of the invention, and a transcriptional and translational termination region functional in plants. The transcriptional initiation region, the promoter, may be native or

analogous or foreign or heterologous to the plant host. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. By "foreign" is intended that the transcriptional initiation region is not found in the native plant into which the transcriptional initiation region is introduced. As used herein, a
5 chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

While it may be preferable to express the sequences using heterologous promoters, the native promoter sequences may be used. Such constructs would change expression levels of RPA in the plant or plant cell. Thus, the phenotype of
10 the plant or plant cell is altered.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline
15 synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272; Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi *et al.* (1987) *Nucleic Acid Res.* 15:9627-9639.

Where appropriate, the gene(s) may be optimized for increased expression
20 in the transformed plant. That is, the genes can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for
25 example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats,
30 and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the

host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *PNAS USA* 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.* (1986); MDMV leader (Maize Dwarf Mosaic Virus); *Virology* 154:9-20), and human immunoglobulin heavy-chain binding protein (BiP), (Macejak *et al.* (1991) *Nature* 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling *et al.* (1987) *Nature* 325:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology* 81:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

The sequences of the present invention can be used to transform or transfect any plant. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained. Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway *et al.* (1986) *Biotechniques* 4:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl.*

- Acad. Sci. USA 83:5602-5606, *Agrobacterium*-mediated transformation (Townsend *et al.*, U.S. Pat No. 5,563,055), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, Sanford *et al.*, U.S. Patent No. 4,945,050; Tomes *et al.*, U.S. Patent No. 5,879,918;
- 5 Tomes *et al.*, U.S. Patent No. 5,886,244; Bidney *et al.*, U.S. Patent No. 5,932,782; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe *et al.* (1988) *Biotechnology* 6:923-926). Also see Weissinger *et al.* (1988)
- 10 *Ann. Rev. Genet.* 22:421-477; Sanford *et al.* (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou *et al.* (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology* 6:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh *et al.* (1998) *Theor. Appl. Genet.* 96:319-324 (soybean); Datta *et al.* (1990)
- 15 *Biotechnology* 8:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology* 6:559-563 (maize); Tomes, U.S. Patent No. 5,240,855; Buising *et al.*, U.S. Patent Nos. 5,322,783 and 5,324,646; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg (Springer-Verlag, Berlin) (maize); Klein *et al.* (1988) *Plant Physiol.* 91:440-444 (maize); Fromm *et al.* (1990) *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren *et al.* (1984) *Nature (London)* 311:763-764; Bowen *et al.*, U.S. Patent No. 5,736,369 (cereals); Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet *et al.* (1985)
- 25 in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports* 9:415-418 and Kaeppler *et al.* (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell* 4:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995)
- 30 *Annals of Botany* 75:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid
5 having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved.

10 Transgenic plants expressing the selectable marker can be screened for transmission of the nucleic acid of the present invention by, for example, standard immunoblot and DNA detection techniques. Transgenic lines are also typically evaluated on levels of expression of the heterologous nucleic acid. Expression at the RNA level can be determined initially to identify and quantitate expression-
15 positive plants. Standard techniques for RNA analysis can be employed and include PCR amplification assays using oligonucleotide primers designed to amplify only the heterologous RNA templates and solution hybridization assays using heterologous nucleic acid-specific probes. The RNA-positive plants can then be analyzed for protein expression by Western immunoblot analysis using the
20 specifically reactive antibodies of the present invention. In addition, *in situ* hybridization and immunocytochemistry according to standard protocols can be done using heterologous nucleic acid specific polynucleotide probes and antibodies, respectively, to localize sites of expression within transgenic tissue. Generally, a number of transgenic lines are usually screened for the incorporated
25 nucleic acid to identify and select plants with the most appropriate expression profiles.

A preferred embodiment is a transgenic plant that is homozygous for the added heterologous nucleic acid; i.e., a transgenic plant that contains two added nucleic acid sequences, one gene at the same locus on each chromosome of a
30 chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) a heterozygous transgenic plant that contains a single added heterologous nucleic acid, germinating some of the seed produced and analyzing the resulting plants produced for altered RPA expression relative to a control plant

(i.e., native, non-transgenic). Backcrossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

The present invention further provides a method for modulating (i.e., increasing or decreasing) RPA levels in a plant or part thereof. Modulation can be effected by increasing or decreasing the total amount of RPA (i.e., its content) and/or the ratio of various RPA subunit proteins (i.e., its composition) in the plant. The method comprises transforming a plant cell with a recombinant expression cassette comprising a polynucleotide of the present invention as described above to obtain a transformed plant cell, growing the transformed plant cell under plant forming conditions, and inducing expression of a polynucleotide of the present invention in the plant for a time sufficient to modulate RPA content and/or composition in the plant or plant part.

In some embodiments, RPA in a plant may be modulated by altering, *in vivo* or *in vitro*, the promoter of a non-isolated RPA gene to up- or down-regulate gene expression. In some embodiments, the coding regions of native RPA genes can be altered via substitution, addition, insertion, or deletion to decrease activity of the encoded enzyme. See, e.g., Kmiec, U.S. Patent 5,565,350; Zarling *et al.*, PCT/US93/03868. And in some embodiments, an isolated nucleic acid (e.g., a vector) comprising a promoter sequence is transfected into a plant cell. Subsequently, a plant cell comprising the promoter operably linked to a polynucleotide of the present invention is selected by means known to those of skill in the art such as, but not limited to, Southern blot, DNA sequencing, or PCR analysis using primers specific to the promoter and to the gene and detecting amplicons produced therefrom. A plant or plant part altered or modified by the foregoing embodiments is grown under plant forming conditions for a time sufficient to modulate RPA content and/or composition in the plant. Plant forming conditions are well known in the art and discussed briefly, *supra*.

In general, content or composition is increased or decreased by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% relative to a native control plant, plant part, or cell lacking the aforementioned recombinant expression cassette. Modulation in the present invention may occur during and/or subsequent to growth of the plant to the desired stage of development. Modulating nucleic acid expression temporally and/or in particular tissues can be controlled by

employing the appropriate promoter operably linked to a polynucleotide of the present invention in, for example, sense or antisense orientation as discussed in greater detail, *supra*. Induction of expression of a polynucleotide of the present invention can also be controlled by exogenous administration of an effective amount of inducing compound. Inducible promoters and inducing compounds that activate expression from these promoters are well known in the art. In preferred embodiments, RPA is modulated in monocots, particularly maize.

The ability of RPA to interact with multiple proteins or protein complexes allows it to participate and regulate these multiple pathways of DNA metabolism.

For example, it has been shown in mammalian systems that RPA interacts with DNA polymerase alpha (Barun *et al.* (1997) *Biochemistry* 36:8443-8454), p53 (Dutta *et al.* (1993) *Nature* 365:79-82), RAD 62 (Park *et al.* (1996) *J. Biol. Chem.* 271:18996-19000).

Participation of the middle subunit of RPA in protein-protein interactions has also been shown. Examples of such interactions include, but are not limited to interactions with XPA protein and RAD 52 (He *et al.* (1995) *Nature* 374:566-69; Matsuda *et al.* (1995) *J. Biol. Chem.* 270:4152-57; Li *et al.* (1995) *Mol. Cell. Biol.* 15:5396-402, Park *et al.* (1996) *J. Biol. Chem.* 271:18996-19000); and PCNA (Shivji *et al.* (1995) *Biochemistry* 34:5011-5017).

Similarly, yeast RPA has been shown to be involved in multiple functions in DNA metabolism (Umezu *et al.* (1998) *Genetics* 148:989-1005). Therefore, the proteins of the invention may be useful as a ligand to purify and clone other proteins involved in DNA recombination, repair, and replication. Particularly, the maize proteins may be useful to purify other maize proteins involved in DNA metabolism. For example, the RPA proteins of the invention may be insolubilized on a solid matrix (e.g. agarose or nylon beads) for affinity purification, or the RPA cDNA may be used as a bait in a yeast two-hybrid system. In this manner, other proteins may be used identified and isolated.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1: cDNA Cloning

Total RNA was isolated from corn tissues with TRIzol Reagent (Life Technology, Inc. Gaithersburg, MD) using a modification of the guanidine isothiocyanate/acid-phenol procedure described by Chomczynski and Sacchi (Chomczynski *et al.* (1987) *Anal. Biochem.* 162:156). In brief, plant tissue samples were pulverized in liquid nitrogen before the addition of the TRIzol Reagent, and then were further homogenized with a mortar and pestle. Addition of chloroform by centrifugation was conducted for separation of an aqueous phase and an organic phase. The total RNA was recovered by precipitation with isopropyl alcohol from the aqueous phase.

The selection of poly(A)+RNA from total RNA was performed using PolyATract system (Promega Corporation, Madison, WI). In brief, biotinylated oligo (dT) primers were used to hybridize to the 3' poly(A) tails on mRNA. The hybrids were captured using streptavidin coupled to paramagnetic particles and a magnetic separation stand. The mRNA was washed at high stringent condition and eluted by Rnase-free deionized water.

Synthesis of the cDNA was performed and unidirectional cDNA libraries were constructed using the SuperScript Plasmid System (Life Technology, Inc., Gaithersburg, MD). First strand of cDNA was synthesized by priming an oligo(dT) primer containing a Not I site. The reaction was catalyzed by SuperScript Reverse Transcriptase II at 45°C. The second strand of cDNA was labeled with α -³²P-dCTP and portions of the molecules smaller than 500 base pairs and unligated adapters were removed by Sephacryl-S400 chromatography. The selected cDNA molecules were ligated into pSPORT1 reference vector between the Not I and Sal I sites.

Individual colonies were picked and DNA was prepared either by PCR with M13 forward primers and M13 reverse primers, or by plasmid miniprep isolation. All the cDNA clones were sequenced using M13 reverse primers.

Two maize homologues for RPA large subunit (ZmRPALSH) have been isolated. The genes map to two different chromosomes as shown below in Table 1.

The amino acid and nucleotide sequences for the two homologues are set forth in SEQ ID NOs: 1-4.

5

Table 1

Maize RPA Large Subunit Genes Map to Two Different Chromosomes

Clone ID	Chromosome No.	Homologue
CBPBS68	c9	ZmRPALSH1
CCRBJ83	c9	ZmRPALSH1
CDPGS47	c9	ZmRPALSH1
CHCLE65	c9	ZmRPALSH1
CJLPL35	c9	ZmRPALSH1
COMGE67	c9	ZmRPALSH1
CBAAK06	c9	ZmRPALSH2
CDPGS46	c9	ZmRPALSH2
CERAG93	c9	ZmRPALSH2
COMFY67	c9	ZmRPALSH2

Ten ESTs, which form two different contigs for maize RPA large subunit, were used as probes for mapping experiments. Each contig represents one maize homologue for RPALS.

10 Seven maize homologues for RPA middle subunit (ZmRPAMSH) have been isolated. The genes map to chromosomes 5 as shown below in Table 2. The nucleotide and amino acid sequences of the seven homologues are set forth in SEQ ID NOs: 11-22.

Table 2

Maize Homologues of Eukaryotic Replication Protein A Middle Subunit

Clone ID	Homologue	Library	Map Position
CCRBK63	ZmRPAMSH-1	P0026	C5
CGEYZ26	ZmRPAMSH-2	P0002	TBD
CGEVJ74	ZmRPAMSH-3	P0002	TBD
CHSBX01	ZmRPABMS-4	P0118	C5
CIMME04	ZmRPAMSH-5	P0114	C5
CRTBB78	ZmRPAMSH-6	P0041	C5
CVRAP89	ZmRPAMSH-7	P0057	C5

5 TBD = To be determined.

Example 2: Transformation and Regeneration of Transgenic Plants:

Immature maize embryos from greenhouse donor plants are bombarded with a plasmid containing the RPA antisense sequence of the invention operably
 10 linked to a pathogen-inducible promoter (Figure 2) plus a plasmid containing the selectable marker gene PAT (Wohlleben *et al.* (1988) *Gene* 70:25-37) that confers resistance to the herbicide Bialaphos. Transformation is performed as follows. All media recipes are in the Appendix.

15 Preparation of Target Tissue

The ears are surface sterilized in 30% Chlorox bleach plus 0.5% Micro detergent for 20 minutes, and rinsed two times with sterile water. The immature embryos are excised and placed embryo axis side down (scutellum side up), 25 embryos per plate, on 560Y medium for 4 hours and then aligned within the 2.5-
 20 cm target zone in preparation for bombardment.

Preparation of DNA

A plasmid vector comprising the RPA sequence of the invention operably linked to a ubiquitin promoter is made. This plasmid DNA plus plasmid DNA containing a PAT selectable marker is precipitated onto 1.1 μm (average diameter) tungsten pellets using a CaCl_2 precipitation procedure as follows:

- 100 μl prepared tungsten particles in water
- 10 μl (1 μg) DNA in TrisEDTA buffer (1 μg total)
- 100 μl 2.5 M CaCl_2
- 10 10 μl 0.1 M spermidine

Each reagent is added sequentially to the tungsten particle suspension, while maintained on the multitube vortexer. The final mixture is sonicated briefly and allowed to incubate under constant vortexing for 10 minutes. After the precipitation period, the tubes are centrifuged briefly, liquid removed, washed with 500 ml 100% ethanol, and centrifuged for 30 seconds. Again the liquid is removed, and 105 μl 100% ethanol is added to the final tungsten particle pellet. For particle gun bombardment, the tungsten/DNA particles are briefly sonicated and 10 μl spotted onto the center of each macrocarrier and allowed to dry about 2 minutes before bombardment.

Particle Gun Treatment

The sample plates are bombarded at level #4 in particle gun #HE34-1 or #HE34-2. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA.

Subsequent Treatment

Following bombardment, the embryos are kept on 560Y medium for 2 days, then transferred to 560R selection medium containing 3 mg/liter Bialaphos, and subcultured every 2 weeks. After approximately 10 weeks of selection, selection-resistant callus clones are transferred to 288J medium to initiate plant regeneration. Following somatic embryo maturation (2-4 weeks), well-developed

- somatic embryos are transferred to medium for germination and transferred to the lighted culture room. Approximately 7-10 days later, developing plantlets are transferred to 272V hormone-free medium in tubes for 7-10 days until plantlets are well established. Plants are then transferred to inserts in flats (equivalent to 2.5”
- 5 pot) containing potting soil and grown for 1 week in a growth chamber, subsequently grown an additional 1-2 weeks in the greenhouse, then transferred to classic 600 pots (1.6 gallon) and grown to maturity. Plants are monitored and scored for expression of the RPA gene of interest.

APPENDIX

272 V

Ingredient	Amount	Unit
D-I H ₂ O	950.000	MI
MS Salts (GIBCO 11117-074)	4.300	G
Myo-Inositol	0.100	G
MS Vitamins Stock Solution ##	5.000	MI
Sucrose	40.000	G
Bacto-Agar @	6.000	G

Directions:

- 5 @ = Add after bringing up to volume

Dissolve ingredients in polished D-I H₂O in sequence

Adjust to pH 5.6

Bring up to volume with polished D-I H₂O after adjusting pH

Sterilize and cool to 60°C.

- 10 ## = Dissolve 0.100 g of Nicotinic Acid; 0.020 g of Thiamine.HCL; 0.100 g of Pyridoxine.HCL; and 0.400 g of Glycine in 875.00 ml of polished D-I H₂O in sequence. Bring up to volume with polished D-I H₂O. Make in 400 ml portions. Thiamine.HCL & Pyridoxine.HCL are in Dark Desiccator. Store for one month, unless contamination or precipitation occurs, then make fresh stock.

- 15 Total Volume (L) = 1.00

288 J

Ingredient	Amount	Unit
D-I H ₂ O	950.000	ml
MS Salts	4.300	G
Myo-Inositol	0.100	G
MS Vitamins Stock Solution ##	5.000	ml
Zeatin .5mg/ml	1.000	ml
Sucrose	60.000	G
Gelrite @	3.000	G
Indoleacetic Acid 0.5 mg/ml #	2.000	ml
0.1mM Absciscic Acid	1.000	ml
Bialaphos 1mg/ml #	3.000	ml

Directions:

@ = Add after bringing up to volume

- 5 Dissolve ingredients in polished D-I H₂O in sequence

Adjust to pH 5.6

Bring up to volume with polished D-I H₂O after adjusting pH

Sterilize and cool to 60°C.

Add 3.5g/L of Gelrite for cell biology.

- 10 ## = Dissolve 0.100 g of Nicotinic Acid; 0.020 g of Thiamine.HCL; 0.100 g of Pyridoxine.HCL; and 0.400 g of Glycine in 875.00 ml of polished D-I H₂O in sequence. Bring up to volume with polished D-I H₂O. Make in 400 ml portions. Thiamine.HCL & Pyridoxine.HCL are in Dark Desiccator. Store for one month, unless contamination or precipitation occurs, then make fresh stock.

- 15 Total Volume (L) = 1.00

560 R

Ingredient	Amount	Unit
D-I Water, Filtered	950.000	MI
CHU (N6) Basal Salts (SIGMA C-1416)	4.000	G
Eriksson's Vitamin Mix (1000X SIGMA-1511)	1.000	MI
Thiamine.HCL 0.4mg/ml	1.250	MI
Sucrose	30.000	G
2, 4-D 0.5mg/ml	4.000	MI
Gelrite @	3.000	G
Silver Nitrate 2mg/ml #	0.425	MI
Bialaphos 1mg/ml #	3.000	MI

Directions:

5 @ = Add after bringing up to volume

= Add after sterilizing and cooling to temp.

Dissolve ingredients in D-I H₂O in sequence

Adjust to pH 5.8 with KOH

Bring up to volume with D-I H₂O

10 Sterilize and cool to room temp.

Total Volume (L) = 1.00

560 Y

Ingredient	Amount	Unit
D-I Water, Filtered	950.000	MI
CHU (N6) Basal Salts (SIGMA C-1416)	4.000	G
Eriksson's Vitamin Mix (1000X SIGMA-1511)	1.000	MI
Thiamine.HCL 0.4mg/ml	1.250	MI
Sucrose	120.000	G
2,4-D 0.5mg/ml	2.000	MI
L-Proline	2.880	G
Gelrite @	2.000	G
Silver Nitrate 2mg/ml #	4.250	MI

Directions:

@ = Add after bringing up to volume

5 # = Add after sterilizing and cooling to temp.

Dissolve ingredients in D-I H₂O in sequence

Adjust to pH 5.8 with KOH

Bring up to volume with D-I H₂O

Sterilize and cool to room temp.

10 ** Autoclave less time because of increased sucrose**

Total Volume (L) = 1.00

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

Applicant's or agent's file reference	5718-59-1	International application No.	PCT/US99/
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 5, lines 5, 8 and 13	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depository institution <p style="text-align: center;">American Type Culture Collection</p>	
Address of depository institution (including postal code and country) <p style="text-align: center;">10801 University Blvd. Manassas, VA 20110-2209 USA</p>	
Date of deposit <p style="text-align: center;">21 August 1998 (21.08.98)</p>	Accession Number <p style="text-align: center;">98843</p>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
Accession No. 98754 - page 5, lines 5, 8 and 13 - Date of deposit : 26 May 1998 (26.05.98)	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indicators are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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THAT WHICH IS CLAIMED:

1. An isolated protein having the amino acid sequence selected from the group consisting of:
 - a) an amino acid sequence of a maize replication protein A large subunit;
 - b) an amino acid an amino acid sequence of a plant replication protein A middle subunit;
 - c) an amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, and SEQ ID NO: 22;
 - d) an amino acid sequence having substantial identity to an amino acid sequence of a), b), or c);
 - e) an amino acid sequence comprising at least 20 contiguous residues of an amino acid sequence of a), b, or c);
 - f) a variant of an amino acid sequence of a), b, or c).
2. An isolated nucleotide sequence selected from the group consisting of:
 - a) a nucleotide sequence encoding a maize replication protein A (RPA) large subunit;
 - b) a nucleotide sequence encoding a plant replication protein A (RPA) middle subunit;
 - c) a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21;
 - d) a nucleotide sequence comprising at least 20 contiguous nucleotides of a nucleotide sequence of a) b), or c);
 - e) an antisense nucleotide sequence corresponding to a nucleotide sequence of a), b), or c);
 - f) a nucleotide sequence that hybridizes to the nucleotide sequences of a), b), or c) under stringent conditions; and

g) a nucleotide sequence that encodes an amino acid sequence according to claim 1.

3. A DNA construct comprising a nucleotide sequence according to claim 2 wherein said nucleotide sequence is operably linked to a promoter that drives expression in a plant cell.

4. The DNA construct of claim 3, wherein said promoter is a tissue-preferred promoter.

10

5. The DNA construct of claim 4, wherein said promoter is a pathogen-inducible promoter.

6. The DNA construct of claim 5, wherein said nucleotide sequence is an antisense sequence.

15

7. The DNA construct of claim 3, wherein said promoter is a constitutive promoter.

8. A method for enhancing homologous recombination in a plant cell, said method comprising transforming said plant cell with at least one nucleotide sequence operably linked to a heterologous promoter that drives expression in a plant cell, said nucleotide sequence selected from the group consisting of:

20

a) a nucleotide sequence encoding a maize replication protein A (RPA) large subunit;

25

b) a nucleotide sequence encoding a plant replication protein A (RPA) middle subunit;

c) a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21;

30

d) a nucleotide sequence comprising at least 20 contiguous nucleotides of a nucleotide sequence of a) b), or c);

- e) a nucleotide sequence that hybridizes to the nucleotide sequences of a), b), or c) under stringent conditions; and
- f) a nucleotide sequence that encodes an amino acid sequence according to claim 1.

5

9. The method of claim 8, wherein said promoter is a constitutive promoter.

10. The method of claim 9, wherein said promoter is an ubiquitin promoter.

11. A method for increasing pathogen resistance in a plant cell, method comprising transforming said plant cell with at least one nucleotide sequence operably linked to a pathogen-inducible promoter said nucleotide sequence selected from the group consisting of:

- a) an antisense nucleotide sequence corresponding to a maize replication protein A large subunit, and
- b) an antisense nucleotide sequence corresponding to a plant replication protein A middle subunit.

20

12. A transformed plant cell having stably incorporated into its genome at least one nucleotide sequence, said nucleotide sequence operably linked to a heterologous promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

- a) a nucleotide sequence encoding a maize replication protein A (RPA) large subunit;
- b) a nucleotide sequence encoding a plant replication protein A (RPA) middle subunit;
- c) a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21;
- d) a nucleotide sequence comprising at least 20 contiguous nucleotides of a nucleotide sequence of a) b), or c);

- e) an antisense nucleotide sequence corresponding to a nucleotide sequence of a), b), or c);
- f) a nucleotide sequence that hybridizes to the nucleotide sequences of a), b), or c) under stringent conditions; and
- 5 g) a nucleotide sequence that encodes an amino acid sequence according to claim 1.

13. A transformed plant having stably incorporated into its genome at least one nucleotide sequence, said nucleotide sequence operably linked
 10 to a heterologous promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

- a) a nucleotide sequence encoding a maize replication protein A (RPA) large subunit;
- b) a nucleotide sequence encoding a plant replication protein A
 15 (RPA) middle subunit;
- c) a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21;
- d) a nucleotide sequence comprising at least 20 contiguous
 20 nucleotides of a nucleotide sequence of a) b), or c);
- e) an antisense nucleotide sequence corresponding to a nucleotide sequence of a), b), or c);
- f) a nucleotide sequence that hybridizes to the nucleotide sequences of a), b), or c) under stringent conditions; and
- 25 g) a nucleotide sequence that encodes an amino acid sequence according to claim 1.

14. Seed of the plant of claim 13.

30 15. The plant claim 13, wherein said plant is a monocot.

16. The plant of claim 15, wherein said monocot is maize, wheat, rice, barley, sorghum, or rye.

17. The plant of claim 13, wherein said plant is a dicot.
18. The plant of claim 17, wherein said dicot is selected from the group
5 consisting of soybean, canola, sunflower, alfalfa, or safflower.
19. Seed of the plant of claim 17.
20. A method for modulating DNA metabolism in a plant cell, said
10 method comprising transforming said plant cell with at least one nucleotide
sequence operably linked to a promoter wherein said nucleotide sequence is
selected from the group consisting of:
- a) a nucleotide sequence encoding a maize replication protein
A (RPA) large subunit;
 - 15 b) a nucleotide sequence encoding a plant replication protein A
(RPA) middle subunit;
 - c) a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID
NO: 3, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ
ID NO: 19, and SEQ ID NO: 21;
 - 20 d) a nucleotide sequence comprising at least 20 contiguous
nucleotides of a nucleotide sequence of a) b), or c);
 - e) an antisense nucleotide sequence corresponding to a
nucleotide sequence of a), b), or c);
 - f) a nucleotide sequence that hybridizes to the nucleotide
25 sequences of a), b), or c) under stringent conditions; and
 - g) a nucleotide sequence that encodes an amino acid sequence
according to claim 1.
21. A method for influencing cell cycle in a plant cell, said method
30 comprising transforming said plant cell with at least one nucleotide sequence
operably linked to a promoter wherein said nucleotide sequence is selected from
the group consisting of:

- a) a nucleotide sequence encoding a maize replication protein A (RPA) large subunit;
- b) a nucleotide sequence encoding a plant replication protein A (RPA) middle subunit;
- 5 c) a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 21;
- d) a nucleotide sequence comprising at least 20 contiguous nucleotides of a nucleotide sequence of a) b), or c);
- 10 e) an antisense nucleotide sequence corresponding to a nucleotide sequence of a), b), or c);
- f) a nucleotide sequence that hybridizes to the nucleotide sequences of a), b), or c) under stringent conditions; and
- g) a nucleotide sequence that encodes an amino acid sequence
- 15 according to claim 1.

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	1				50
ZMRPALSH1	~~MDAAKSVT	PGAVSYIL..	AHPSTGSDGA	VSDLVVQVLD	LKSIGMGS.R
ZMRPALSH2	~~MDAAKLVT	PVAVSHIL..	AHPSAGSDGA	VTDLVVQVLD	LKSVGTGS.R
024183	MDSDAAPSVT	PGAVAFVLEN	ASPDAAATGVP	VPEIVLQVVD	LKPIGT...R
Rfal_Xenla	~~~MALPQLS	EGAISA-MLG	GDSSC..KPT	LQVINIRPIN	...TGNGPPR
Rfal_Human	~~~~MVGQLS	EGAIAAIMQK	GDTNI..KPI	LQVINIRPIT	...TGNSPPR
Rfal_Drome	~~~MVLASLS	TGVIARIM.H	GEVVD..APV	LQILAIKKIN	...SAADSER
Rfal_Schpo	~~~~MAERLS	VGALRIINTS	DASSFPNPPI	LQVLTVKELN	SNPTSGAPKR
Rfal_Yeast	~~~MSSVQLS	RGDFHSIFTN	KQR..YDNPT	GGVYQVYNTR	KSDGANSNRK
	51				100
ZMRPALSH1	FSFTASDGND	KIKA.MLPTY	FASEVHSGNL	KNFGLIRILD	YTCNSVK..G
ZMRPALSH2	FSFTATDGKD	KIKA.MLPTN	FGSEVRSGNL	KNLGLIRIID	YTCNVVK..G
024183	FTFLASDGKD	KIKT.MLLTQ	LAPEVRSGNI	QNLGVIRVLD	YTCNTIG..E
Rfal_Xenla	YRLLMSDGLN	TLSSFMLATQ	LNSLVDNLL	ATNCICQVSR	FIVNNL.KD.
Rfal_Human	YRLLMSDGLN	TLSSFMLATQ	LNPLVEEEQL	SSNCVCQIHR	FIVNTL.KD.
Rfal_Drome	YRILISDGKY	FNSYAMLASQ	LNVMQHNGEL	EEFTIVQLDK	YVTSLVGKDG
Rfal_Schpo	YRVVLSDSIN	YAQS.MLSTQ	LNHLVAENKL	QKGAGVQLTQ	FTVNVME..
Rfal_Yeast	NLIMISDGIY	HMKA.LLRNQ	AASKFQSMEL	QRGDIIRV..	IIAEPAIRRE
	101				150
ZMRPALSH1	NADKVLIVVK	CETVCEA..L	DAEINGEAKK	ED..PPIVLK	PKDEGSVVAE
ZMRPALSH2	KDDKVLVVIK	CELVCQA..L	DAEINGEAKK	EE..PPIVLK	PKDECYGV..
024183	KQEKVLIITK	LEVVFKA..L	DSEIKCEAEK	QEEKPAILLS	PKEESVVLŠK
Rfal_Xenla	.GRRVIVME	LDVLKSADLV	MKGIGNPQPY	ND..GQPQPA	APAPASAPA.
Rfal_Human	.GRRVVILME	LEVLSAEAV	GVKIGNPVPY	NEGLGQPQVA	PPAPAASPAA
Rfal_Drome	AGKRVLIISE	LTVVNPGAEV	KSKIGEPVTY	ENAAKQDLAP	KPAVTSNSKP
Rfal_Schpo	.RKILIVLG	LNVLTELG.V	MDKIGNPAGL	ETVDALRQQQ	NEQNNASAPR
Rfal_Yeast	RKKYVLLVDD	FELVQSRADM	VNQTSTFLDN	YFSEHPNETL	KDEDITDSGN
	151				200
ZMRPALSH1	ETNSPP..L.	..VMKPKQEV	KSASQIVTEQ	RGNAAPATRL	SMTRRVHPLI
ZMRPALSH2	...TSP..L.	..VMKPKQEV	KSASQIVTEQ	RGNAAPATRL	SMTRRVHPLI
024183	PTNAPP..LP	PVVLKPKQEV	KSASQIVNEQ	RGNAAPAARL	AMTRRVHPLI
Rfal_Xenla	.PAPSKLQ	NNSAPPPSMN	RGTSKLFG..	.GGSLLNTPG	GSQSKVVPJA
Rfal_Human	ŠSRPQPQNGS	SGMGSTVSKA	YGASKTFGKA	AGPSLSHTSG	GTQSKVVPJA
Rfal_Drome	IAKKEPSHNN	NN..	..NIVMNSS	INSGMTHPIS	..STIIYPIE
Rfal_Schpo	TGISTSTNSF	YGNNAAATAP	ÄPPPMKKPA	ÄPNSL..	..STIIYPIE
Rfal_Yeast	VA...NQTN	ASNAGVPDML	HSNSNLNANE	RKFANENPNŠ	QKTRPIFAIE
	201				250
ZMRPALSH1	TLNPYQGNWV	IKVRVTSKGN	LRTYRNARGE	GCVFNVELTD	EDGTQIQATM
ZMRPALSH2	TLNPYQGNWV	IKVRVTSKGN	LRTYRNARGE	GCVFNVELTD	EDGTQIQATM
024183	SLNPYQGNWI	IKVRVTSKGN	LRTYKNARGE	GCVFNVELTD	VDGTQIQATM
Rfal_Xenla	SLNPYQSKWT	VRARVTNKGG	IRTWSNRGE	GKLFSIEMVD	ESG EIRATA
Rfal_Human	SLTPYQSKWT	ICARVTNKSQ	IRTWSNRGE	GKLFSLELVD	ESG EIRATA
Rfal_Drome	SLSPYQNKWV	IKARVTSKSG	IRTWSNARGE	GKLFSMDLMD	ESG EIRATA
Rfal_Schpo	GLSPYQNKWT	IRARVTNKSE	VKHWNNQRGE	GKLFSVNLLD	ESG EIRATG
Rfal_Yeast	QLSPYQNVWT	IKARVSYKGE	IKTWHNNQRGD	GKLFNVNFLD	TSG EIRATA

TO FIG. 1B.

Comparison of eukaryotic RPA LS amino acid sequences

FIG. 1A.

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FROM FIG. 1A.

	251				300
ZMRPALSH1	FNEAAKKFYF	IFELGKVVYV	SKGSLRIANK	QFKTVKNDYE	LSLNENAIVE
ZMRPALSH2	FNDAAKKFYP	IFELGKVVYV	SKGSLRIANK	QFKTVQNDYE	MSLNENAIVE
024183	FNEAAKKFYF	MFELGKVVYI	SKGSLRVANK	QFKTVHNDYE	MTLNENAVVE
Rfal_Xenla	FNEQADKFFS	IEVNKVVYF	SKGTLKIANK	QYTSVKNDYE	MTFNSETSVI
Rfal_Human	FNEQVDKFFP	LIEVNKVVYF	SKGTLKIANK	QFTAVKNDYE	MTFNNETSVM
Rfal_Drome	FKEQCDKFYD	LIQVDSVYI	SKCQLKPANK	QYSSLNNAYE	MTFSGETTVQ
Rfal_Schpo	FNDQVDAFYD	ILQEGSVYI	SRCRVNIACK	QYTNVQNEYE	LMFERDTEIR
Rfal_Yeast	FNDFATKFNE	ILQEGKVVYV	SKAKLQPAKP	QFTNLTHPYE	LNLDRDTVIE
	301				350
ZMRPALSH1	EAE..GETFL	PPVQYNLVKI	DQLGPYVGGR	ELVDIVGVVQ	SVSPTLSVRR
ZMRPALSH2	EAE..GETCI	PQVQYNLVKI	DQLGSYVGGR	ELVDIVGVVQ	SVSPTLSVRR
024183	EAE..GETFI	PQIQYNFVKI	DQLGPYVGGR	ELVDIVGVVQ	SVSPTLSVRR
Rfal_Xenla	PCDDSD..V	PMVQFEFVSI	GELES.KNKD	TVLDIIGVCK	NVEEVTKVTI
Rfal_Human	PCEDDH..L	PTVQDFDTGI	DDLEN.KSKD	SLVDIIGICK	SYEDATKITY
Rfal_Drome	LCEDTDDPI	PEIKYNLVPI	SDVSG.MENK	AAVDITIGICK	EVGELQSFVA
Rfal_Schpo	KAED..QTAV	PVAKFSFVSL	QEVGD.VAKD	AVIDVIGVLQ	NVGPVQQITS
Rfal_Yeast	ECFDES..V	PKTHFNFIKL	DAIGN.QEVN	SNVDVLGIIQ	TINPHFELTS
	351				400
ZMRPALSH1	KIDNETIPKR	DIVVADDSSGK	TVTISLWNDL	ATTTGQELLD	MVDSSPVVAI
ZMRPALSH2	KIDNETIPKR	DIVVADDSSGK	TVSISLVNDL	ATTTGQELLD	MADSSPVVAI
024183	KIDNETIPKR	DIVVADDSSK	TVTISLWNDL	ATTTGQELLD	MVDSAPIIAI
Rfal_Xenla	KSNNREVS KR	SIHLMDSSGK	VVSTTLWGED	ADKFD.....	.GSRQPVVAI
Rfal_Human	RSNNREVAKR	NIYLMDSGK	VVTATLWGED	ADKFD.....	.GSRQPVVAI
Rfal_Drome	RTTNKEFKKR	DITLVDMSNS	AISLTLWGDD	AVNFD.....	.GHVQPVILV
Rfal_Schpo	RATSRGFDR	DITIVDQGTG	EMRVTLWGKT	AIEFS.....	.VSEESILAF
Rfal_Yeast	RA.GKKFDRR	DITIVDDSGF	SISVGLWNQQ	ALDFN.....	.LPEGSVAAI
	401				450
ZMRPALSH1	KSLKVSDFQ.	GVSLSTIGRS	TLEINPDLPE	AKNLKSWYDS	EGKDTSLAPI
ZMRPALSH2	KSLKVSDFQ.	GVSLSTVGKS	TLAINPDLHE	AQNLKSWYDS	EGKDTSLAPI
024183	KSLKVSDFQ.	GLSLSTVGRS	TIVVNPDLPE	AEQLRAWYDS	EGKGTSMASI
Rfal_Xenla	KGARLSDF.G	GRSLSVLSSS	TVMINPDIPE	AFKLRAWFDS	EGQVVEGTSI
Rfal_Human	KGARVSDF.G	GRSLSVLESSS	TIIANPDIPE	AYKLRGWFDA	EGQALDGVSI
Rfal_Drome	KGTRINEFNG	GKSLSLGGGS	IMKINPDIPE	AHKLRGWF DN	GGGDSVANMV
Rfal_Schpo	KGVKVNDFQ.	GRSLSMLTSS	TMSVDPDIQE	SHLLDGWYDG	QGRGQEF AKH
Rfal_Yeast	KGVRVTD F.G	GKSLSMGFSS	TLIPNPEIPE	AYALKGWYDS	KGRNANFITL
	451				500
ZMRPALSH1	SAEAGATRAG	G..FKSMYSD	RVFLSHITSD	PAMGQEKPVF	FSLYAIISHI
ZMRPALSH2	GAEMGAARAG	G..FKSTYSD	RVFLSHITSD	PAMGQEKPVF	FSLYATISHI
024183	GSDMGASRVG	G..ARSMYSD	RVFLSHITSD	PNLGQDKPVF	FSLNAYISLI
Rfal_Xenla	SESRGG.GTG	GGN.....TN	WKSLLLEV KNE	NLGHGEKADY	FTSVATIVYL
Rfal_Human	SDLKSG.GVG	GSN.....TN	WKTLYEVKSE	NLGGQDKPDY	FSSVATVVYL
Rfal_Drome	SARTGG...G	SFS.....TE	WMTLK DARAR	NLGSQDKPDY	FQKAVVHIV
Rfal_Schpo	SVISSTLSTT	GRS.....AE	RKNIAEVQAE	HLGMSETPDY	FSLKGTIVYI
Rfal_Yeast	KQEPGMGGQS	AASLTKFIAQ	RITIA RAQAE	NLGRSEK GDF	FSVKAAISFL

TO FIG. 1C.

Comparison of eukaryotic RPA LS amino acid sequences

FIG. 1B.

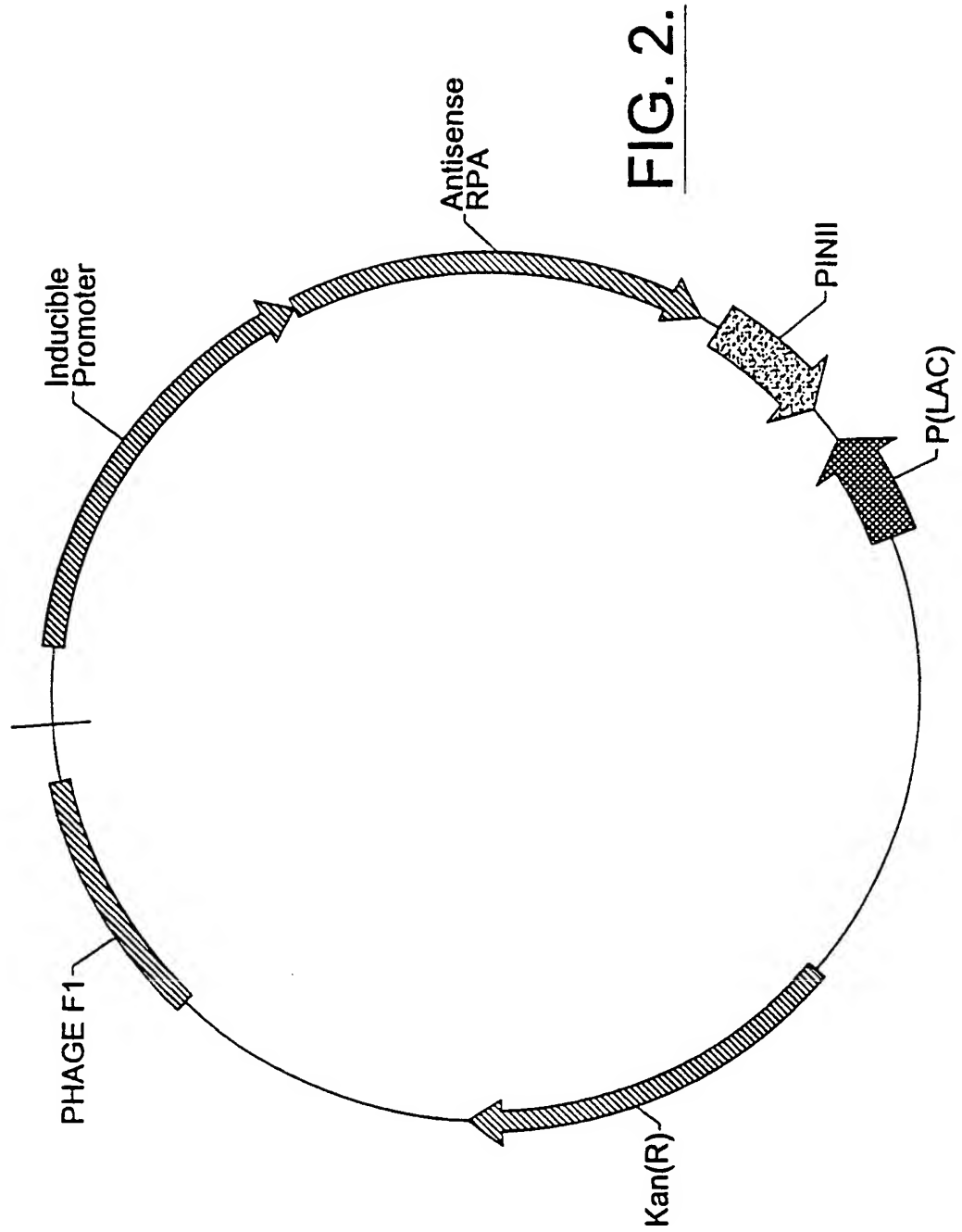
3/4

FROM FIG. 1B.

	501					550
ZMRPALSH1	KPDQNMWYRA	CTT . CNKKV	TEAFGSGYWC	EGCQKNDSEC	SLRYIMVIKL	
ZMRPALSH2	KPDQNMWYRA	CKT . CNKKV	TETFGSGYWC	EGCQKNDSEC	SLRYIMVIKV	
024183	KPDQTMWYRA	CKT . CNKKV	TEAMGSGYWC	EGCQKNDSEC	SLRYIMVIKV	
Rfal_Xenla	RKE . NCLYQA	CPQSDCNKKV	IDQQNGLFRC	EKCNKEFPNF	KYRLILSANI	
Rfal_Human	REK . NCMYQA	OPTQDCNKKV	IDQQNGLYRC	EKCIDTEFPNF	KYRMILSVNI	
Rfal_Drome	KQE . NAFYRA	CPQSDCNKKV	VDECNDQFRC	EKCNALFPNF	KYRLLINMSI	
Rfal_Schpo	RKK . NVSYPA	CPAADCNKKV	FDQC . GSWRC	EKCNKEYDAP	QYRYIITIAV	
Rfal_Yeast	KVD . NFAYPA	CSNENCNKKV	LEQPDGTWRC	EKQDTNNARP	NWRYILTISI	
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Rfal_Xenla	ADFGENQWIT	CFQESAISIL	GQNATYLGEL	.KEKNEQAYD	EVFQANAFRS	
Rfal_Human	ADFGENQWVT	CFQESAIAIL	GQNAAYLGEL	.KDKNEQAFE	EVFQANAFRS	
Rfal_Drome	GDWTSNRWVS	SFNEVGEQLL	GHTSQEVGEA	.LENDPAKAE	QIFSALNFTS	
Rfal_Schpo	GDHTGQLWLN	VFDDVGKLIM	HKTADELNDL	.QENDENAFM	NCMAEACYP	
Rfal_Yeast	IDETNQLWLT	LFDDQAKQLL	GVDANTLMSL	.KEEDPNEFT	KITQSIQMNE	
	601					650
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024183	HLFRVSVTQN	EYMNEKRQRI	TVRSEAPVDH	AAEAKYMLEE	IAKLTGC*~	
Rfal_Xenla	YTFRARVKLE	TYNDESRKA	TAVDVKPVDP	KEYSRRLIMN	IRKMATQGV~	
Rfal_Human	FIFRVRVKVE	TYNDESRKA	TVMDVKPVDP	REYGRRLVMS	IRRSALM*~	
Rfal_Drome	HIFKLRCKNE	VYGDMTNRKL	TVQSVAPINH	KEYNKHLLKE	LQELTGIGSS	
Rfal_Schpo	YIFQCRAKQD	NFKGENRVRY	TVMSINQMDW	KEESKRLINF	IESAQ*~	
Rfal_Yeast	YDFRIRARED	TYNDQSRIRY	TVANLHSLNY	RAEADYLADE	LSKALLA*~	
	651					
ZMRPALSH1	~					
ZMRPALSH2	~					
024183	~					
Rfal_Xenla	~					
Rfal_Human	~					
Rfal_Drome	N					
Rfal_Schpo	~					
Rfal_Yeast	~					

Comparison of eukaryotic RPA LS amino acid sequences

FIG. 1C.



SEQUENCE LISTING

<110> Mahajan, Pramod B.

<120> Maize Replication Protein A and Use

<130> 5718-59

<160> 22

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Met Asp Ala Ala Lys Ser	
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Ser Asp Gly Ala Val Ser Asp Leu Val Val Gln Val Leu Asp Leu Lys	
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Ser Ile Gly Met Gly Ser Arg Phe Ser Phe Thr Ala Ser Asp Gly Asn	
40 45 50	
gac aaa atc aag gcg atg ctc ccc act tac ttt gcg tcg gag gtc cac	366
Asp Lys Ile Lys Ala Met Leu Pro Thr Tyr Phe Ala Ser Glu Val His	
55 60 65 70	
tcc ggc aat ctg aag aat ttc ggt ctc atc cgc atc ctc gac tac act	414
Ser Gly Asn Leu Lys Asn Phe Gly Leu Ile Arg Ile Leu Asp Tyr Thr	
75 80 85	
tgc aac tcc gtc aaa ggc aac gct gac aaa gtc ctg att gtc gtc aaa	462

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Cys	Glu	Thr	Val	Cys	Glu	Ala	Leu	Asp	Ala	Glu	Ile	Asn	Gly	Glu	Ala		
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Lys	Lys	Glu	Asp	Pro	Pro	Ile	Val	Leu	Lys	Pro	Lys	Asp	Glu	Gly	Ser		
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gtc	gtg	gct	gag	gaa	aca	aat	tct	ccc	cca	ctc	gtg	atg	aag	cct	aag	606	
Val	Val	Ala	Glu	Glu	Thr	Asn	Ser	Pro	Pro	Leu	Val	Met	Lys	Pro	Lys		
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Ala	Ala	Pro	Ala	Thr	Arg	Leu	Ser	Met	Thr	Arg	Arg	Val	His	Pro	Leu		
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Gln	Tyr	Asn	Leu	Val	Lys	Ile	Asp	Gln	Leu	Gly	Pro	Tyr	Val	Gly	Gly		
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gtc aca cag cat gaa tac atg aac gag aag agg cag aga atc acc gtg      1950
Val Thr Gln His Glu Tyr Met Asn Glu Lys Arg Gln Arg Ile Thr Val
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agg ggt gaa gca ccg gtc gac ttc gca gct gag tcc aag tac ttg ctt      1998
Arg Gly Glu Ala Pro Val Asp Phe Ala Ala Glu Ser Lys Tyr Leu Leu
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gaa gag atc gcg aag ctc acc gct tgc tagaagacgc agtctttctg      2045
Glu Glu Ile Ala Lys Leu Thr Ala Cys
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tccctatatt ttattctatt ttagtattta aggttgcggt tgggtgcgct gactagacat      2405
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 Thr Ala Ser Asp Gly Asn Asp Lys Ile Lys Ala Met Leu Pro Thr Tyr
 50 55 60
 Phe Ala Ser Glu Val His Ser Gly Asn Leu Lys Asn Phe Gly Leu Ile
 65 70 75 80
 Arg Ile Leu Asp Tyr Thr Cys Asn Ser Val Lys Gly Asn Ala Asp Lys
 85 90 95
 Val Leu Ile Val Val Lys Cys Glu Thr Val Cys Glu Ala Leu Asp Ala
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 Glu Ile Asn Gly Glu Ala Lys Lys Glu Asp Pro Pro Ile Val Leu Lys
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 Pro Lys Asp Glu Gly Ser Val Val Ala Glu Glu Thr Asn Ser Pro Pro
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ccg gtc gct gtg tct cac att ctg gcg cac ccg tcg gcg ggc tcc gac      162
Pro Val Ala Val Ser His Ile Leu Ala His Pro Ser Ala Gly Ser Asp
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ggc gca gtg acc gat ctc gtc gtt cag gtc ctc gac ctg aag tcc gtc      210
Gly Ala Val Thr Asp Leu Val Val Gln Val Leu Asp Leu Lys Ser Val
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ggc acg ggc agc cgg ttc agt ttc aca gca act gac ggg aag gat aag      258
Gly Thr Gly Ser Arg Phe Ser Phe Thr Ala Thr Asp Gly Lys Asp Lys
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atc aag gcg atg ctt ccc acc aac ttc ggg tcg gag gtc cgc tct ggc      306
Ile Lys Ala Met Leu Pro Thr Asn Phe Gly Ser Glu Val Arg Ser Gly
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aac ctg aag aac ctc ggc ctc atc cgc atc atc gac tac act tgc aac      354
Asn Leu Lys Asn Leu Gly Leu Ile Arg Ile Ile Asp Tyr Thr Cys Asn
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   90                               95                               100

ctt gtg tgc caa gcg ctt gac gcc gag atc aac ggc gag gcc aaa aaa      450
Leu Val Cys Gln Ala Leu Asp Ala Glu Ile Asn Gly Glu Ala Lys Lys
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gag gag cct cca att gtg ctg aag cct aag gac gaa tgc gtg ggc gtg      498
Glu Glu Pro Pro Ile Val Leu Lys Pro Lys Asp Glu Cys Val Gly Val
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act tcc cca ctc gct atg aag ccc aag cag gag gtg aag tct gcg tcc      546
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Met Asp Ala Ala Lys Leu Val Thr Pro Val Ala Val Ser His Ile Leu
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 Ala His Pro Ser Ala Gly Ser Asp Gly Ala Val Thr Asp Leu Val Val
 20 25 30
 Gln Val Leu Asp Leu Lys Ser Val Gly Thr Gly Ser Arg Phe Ser Phe
 35 40 45
 Thr Ala Thr Asp Gly Lys Asp Lys Ile Lys Ala Met Leu Pro Thr Asn
 50 55 60
 Phe Gly Ser Glu Val Arg Ser Gly Asn Leu Lys Asn Leu Gly Leu Ile
 65 70 75 80
 Arg Ile Ile Asp Tyr Thr Cys Asn Val Val Lys Gly Lys Asp Asp Lys
 85 90 95
 Val Leu Val Val Ile Lys Cys Glu Leu Val Cys Gln Ala Leu Asp Ala
 100 105 110
 Glu Ile Asn Gly Glu Ala Lys Lys Glu Glu Pro Pro Ile Val Leu Lys
 115 120 125
 Pro Lys Asp Glu Cys Val Gly Val Thr Ser Pro Leu Ala Met Lys Pro
 130 135 140
 Lys Gln Glu Val Lys Ser Ala Ser Gln Ile Val Asn Glu Gln Arg Gly
 145 150 155 160
 Asn Thr Ala Pro Val Lys Pro Leu Ser Met Thr Lys Arg Val His Pro
 165 170 175
 Leu Ile Thr Leu Asn Pro Tyr Gln Gly Asn Trp Val Ile Lys Val Arg
 180 185 190
 Val Thr Ser Lys Gly Asn Leu Arg Thr Tyr Arg Asn Ala Arg Gly Glu
 195 200 205
 Gly Cys Val Phe Asn Val Glu Leu Thr Asp Glu Asp Gly Thr Gln Ile
 210 215 220
 Gln Ala Thr Met Phe Asn Asp Ala Ala Lys Lys Phe Tyr Pro Ile Phe
 225 230 235 240
 Glu Leu Gly Lys Val Tyr Tyr Val Ser Lys Gly Ser Leu Arg Ile Ala
 245 250 255
 Asn Lys Gln Phe Lys Thr Val Gln Asn Asp Tyr Glu Met Ser Leu Asn
 260 265 270
 Glu Asn Ala Ile Val Glu Glu Ala Glu Gly Glu Thr Cys Ile Pro Gln
 275 280 285
 Val Gln Tyr Asn Leu Val Lys Ile Asp Gln Leu Gly Ser Tyr Val Gly
 290 295 300
 Gly Arg Glu Leu Val Asp Ile Val Gly Val Val Gln Ser Val Ser Pro

```

305          310          315          320
Thr Leu Ser Val Arg Arg Lys Ile Asp Asn Glu Thr Ile Pro Lys Arg
          325          330          335
Asp Ile Val Val Ala Asp Asp Ser Gly Lys Thr Val Ser Ile Ser Leu
          340          345          350
Trp Asn Asp Leu Ala Thr Thr Thr Gly Gln Glu Leu Leu Asp Met Ala
          355          360          365
Asp Ser Ser Pro Val Val Ala Ile Lys Ser Leu Lys Val Ser Asp Phe
          370          375          380
Gln Gly Val Ser Leu Ser Thr Val Gly Lys Ser Thr Leu Ala Ile Asn
385          390          395          400
Pro Asp Leu His Glu Ala Gln Asn Leu Lys Ser Trp Tyr Asp Ser Glu
          405          410          415
Gly Lys Asp Thr Ser Leu Ala Pro Ile Gly Ala Glu Met Gly Ala Ala
          420          425          430
Arg Ala Gly Gly Phe Lys Ser Thr Tyr Ser Asp Arg Val Phe Leu Ser
          435          440          445
His Ile Thr Ser Asp Pro Ala Met Gly Gln Glu Lys Pro Val Phe Phe
          450          455          460
Ser Leu Tyr Ala Thr Ile Ser His Ile Lys Pro Asp Gln Asn Met Trp
465          470          475          480
Tyr Arg Ala Cys Lys Thr Cys Asn Lys Lys Val Thr Glu Thr Phe Gly
          485          490          495
Ser Gly Tyr Trp Cys Glu Gly Cys Gln Lys Asn Asp Ser Glu Cys Ser
          500          505          510
Leu Arg Tyr Ile Met Val Ile Lys Val Ser Asp Pro Thr Gly Glu Ala
          515          520          525
Trp Phe Ser Val Phe Asn Glu His Ala Glu Lys Ile Ile Gly Cys Ser
          530          535          540
Ala Asp Glu Leu Asp Arg Ile Arg Lys Glu Glu Gly Asp Asp Ser Tyr
545          550          555          560
Val Leu Lys Leu Lys Glu Ala Thr Trp Val Pro His Leu Phe Arg Val
          565          570          575
Ser Val Thr Gln His Glu Tyr Asn Asn Glu Lys Arg Gln Arg Ile Thr
          580          585          590
Val Arg Ser Glu Ala Pro Val Glu His Ala Ala Glu Ser Lys Tyr Leu
          595          600          605
Leu Glu Gln Ile Ala Lys Leu Thr Ala
610          615

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<210> 5
<211> 630
<212> PRT
<213> Oryza sativa

```

```

<400> 5
Met Asp Ser Asp Ala Ala Pro Ser Val Thr Pro Gly Ala Val Ala Phe
1          5          10          15
Val Leu Glu Asn Ala Ser Pro Asp Ala Ala Thr Gly Val Pro Val Pro
20          25          30
Glu Ile Val Leu Gln Val Val Asp Leu Lys Pro Ile Gly Thr Arg Phe
35          40          45
Thr Phe Leu Ala Ser Asp Gly Lys Asp Lys Ile Lys Thr Met Leu Leu
50          55          60
Thr Gln Leu Ala Pro Glu Val Arg Ser Gly Asn Ile Gln Asn Leu Gly
65          70          75          80
Val Ile Arg Val Leu Asp Tyr Thr Cys Asn Thr Ile Gly Glu Lys Gln

```

11

Phe Asn Asp Gln Ala Glu Arg Ile Val Gly Cys Ser Ala Asp Glu Leu
 545 550 555 560
 Asp Arg Ile Arg Lys Glu Glu Gly Asp Asp Ser Tyr Leu Leu Lys Leu
 565 570 575
 Lys Glu Ala Thr Trp Val Pro His Leu Phe Arg Val Ser Val Thr Gln
 580 585 590
 Asn Glu Tyr Met Asn Glu Lys Arg Gln Arg Ile Thr Val Arg Ser Glu
 595 600 605
 Ala Pro Val Asp His Ala Ala Glu Ala Lys Tyr Met Leu Glu Glu Ile
 610 615 620
 Ala Lys Leu Thr Gly Cys
 625 630

<210> 6
 <211> 609
 <212> PRT
 <213> Xenopus laevis

<400> 6
 Met Ala Leu Pro Gln Leu Ser Glu Gly Ala Ile Ser Ala Met Leu Gly
 1 5 10 15
 Gly Asp Ser Ser Cys Lys Pro Thr Leu Gln Val Ile Asn Ile Arg Pro
 20 25 30
 Ile Asn Thr Gly Asn Gly Pro Pro Arg Tyr Arg Leu Leu Met Ser Asp
 35 40 45
 Gly Leu Asn Thr Leu Ser Ser Phe Met Leu Ala Thr Gln Leu Asn Ser
 50 55 60
 Leu Val Asp Asn Asn Leu Leu Ala Thr Asn Cys Ile Cys Gln Val Ser
 65 70 75 80
 Arg Phe Ile Val Asn Asn Leu Lys Asp Gly Arg Arg Val Ile Ile Val
 85 90 95
 Met Glu Leu Asp Val Leu Lys Ser Ala Asp Leu Val Met Gly Lys Ile
 100 105 110
 Gly Asn Pro Gln Pro Tyr Asn Asp Gly Gln Pro Gln Pro Ala Ala Pro
 115 120 125
 Ala Pro Ala Ser Ala Pro Ala Pro Ala Pro Ser Lys Leu Gln Asn Asn
 130 135 140
 Ser Ala Pro Pro Pro Ser Met Asn Arg Gly Thr Ser Lys Leu Phe Gly
 145 150 155 160
 Gly Gly Ser Leu Leu Asn Thr Pro Gly Gly Ser Gln Ser Lys Val Val
 165 170 175
 Pro Ile Ala Ser Leu Asn Pro Tyr Gln Ser Lys Trp Thr Val Arg Ala
 180 185 190
 Arg Val Thr Asn Lys Gly Gln Ile Arg Thr Trp Ser Asn Ser Arg Gly
 195 200 205
 Glu Gly Lys Leu Phe Ser Ile Glu Met Val Asp Glu Ser Gly Glu Ile
 210 215 220
 Arg Ala Thr Ala Phe Asn Glu Gln Ala Asp Lys Phe Phe Ser Ile Ile
 225 230 235 240
 Glu Val Asn Lys Val Tyr Tyr Phe Ser Lys Gly Thr Leu Lys Ile Ala
 245 250 255
 Asn Lys Gln Tyr Thr Ser Val Lys Asn Asp Tyr Glu Met Thr Phe Asn
 260 265 270
 Ser Glu Thr Ser Val Ile Pro Cys Asp Asp Ser Ala Asp Val Pro Met
 275 280 285
 Val Gln Phe Glu Phe Val Ser Ile Gly Glu Leu Glu Ser Lys Asn Lys
 290 295 300

Asp Thr Val Leu Asp Ile Ile Gly Val Cys Lys Asn Val Glu Glu Val
 305 310 315 320
 Thr Lys Val Thr Ile Lys Ser Asn Asn Arg Glu Val Ser Lys Arg Ser
 325 330 335
 Ile His Leu Met Asp Ser Ser Gly Lys Val Val Ser Thr Thr Leu Trp
 340 345 350
 Gly Glu Asp Ala Asp Lys Phe Asp Gly Ser Arg Gln Pro Val Val Ala
 355 360 365
 Ile Lys Gly Ala Arg Leu Ser Asp Phe Gly Gly Arg Ser Leu Ser Val
 370 375 380
 Leu Ser Ser Ser Thr Val Met Ile Asn Pro Asp Ile Pro Glu Ala Phe
 385 390 395 400
 Lys Leu Arg Ala Trp Phe Asp Ser Glu Gly Gln Val Val Glu Gly Thr
 405 410 415
 Ser Ile Ser Glu Ser Arg Gly Gly Gly Thr Gly Gly Gly Asn Thr Asn
 420 425 430
 Trp Lys Ser Leu Leu Glu Val Lys Asn Glu Asn Leu Gly His Gly Glu
 435 440 445
 Lys Ala Asp Tyr Phe Thr Ser Val Ala Thr Ile Val Tyr Leu Arg Lys
 450 455 460
 Glu Asn Cys Leu Tyr Gln Ala Cys Pro Ser Gln Asp Cys Asn Lys Lys
 465 470 475 480
 Val Ile Asp Gln Gln Asn Gly Leu Phe Arg Cys Glu Lys Cys Asn Lys
 485 490 495
 Glu Phe Pro Asn Phe Lys Tyr Arg Leu Ile Leu Ser Ala Asn Ile Ala
 500 505 510
 Asp Phe Gly Glu Asn Gln Trp Ile Thr Cys Phe Gln Glu Ser Ala Glu
 515 520 525
 Ser Ile Leu Gly Gln Asn Ala Thr Tyr Leu Gly Glu Leu Lys Glu Lys
 530 535 540
 Asn Glu Gln Ala Tyr Asp Glu Val Phe Gln Asn Ala Asn Phe Arg Ser
 545 550 555 560
 Tyr Thr Phe Arg Ala Arg Val Lys Leu Glu Thr Tyr Asn Asp Glu Ser
 565 570 575
 Arg Ile Lys Ala Thr Ala Val Asp Val Lys Pro Val Asp His Lys Glu
 580 585 590
 Tyr Ser Arg Arg Leu Ile Met Asn Ile Arg Lys Met Ala Thr Gln Gly
 595 600 605
 Val

<210> 7

<211> 616

<212> PRT

<213> Homo sapiens

<400> 7

Met Val Gly Gln Leu Ser Glu Gly Ala Ile Ala Ala Ile Met Gln Lys
 1 5 10 15
 Gly Asp Thr Asn Ile Lys Pro Ile Leu Gln Val Ile Asn Ile Arg Pro
 20 25 30
 Ile Thr Thr Gly Asn Ser Pro Pro Arg Tyr Arg Leu Leu Met Ser Asp
 35 40 45
 Gly Leu Asn Thr Leu Ser Ser Phe Met Leu Ala Thr Gln Leu Asn Pro
 50 55 60
 Leu Val Glu Glu Glu Gln Leu Ser Ser Asn Cys Val Cys Gln Ile His
 65 70 75 80

Arg Phe Ile Val Asn Thr Leu Lys Asp Gly Arg Arg Val Val Ile Leu
 85 90 95
 Met Glu Leu Glu Val Leu Lys Ser Ala Glu Ala Val Gly Val Lys Ile
 100 105 110
 Gly Asn Pro Val Pro Tyr Asn Glu Gly Leu Gly Gln Pro Gln Val Ala
 115 120 125
 Pro Pro Ala Pro Ala Ala Ser Pro Ala Ala Ser Ser Arg Pro Gln Pro
 130 135 140
 Gln Asn Gly Ser Ser Gly Met Gly Ser Thr Val Ser Lys Ala Tyr Gly
 145 150 155 160
 Ala Ser Lys Thr Phe Gly Lys Ala Ala Gly Pro Ser Leu Ser His Thr
 165 170 175
 Ser Gly Gly Thr Gln Ser Lys Val Val Pro Ile Ala Ser Leu Thr Pro
 180 185 190
 Tyr Gln Ser Lys Trp Thr Ile Cys Ala Arg Val Thr Asn Lys Ser Gln
 195 200 205
 Ile Arg Thr Trp Ser Asn Ser Arg Gly Glu Gly Lys Leu Phe Ser Leu
 210 215 220
 Glu Leu Val Asp Glu Ser Gly Glu Ile Arg Ala Thr Ala Phe Asn Glu
 225 230 235 240
 Gln Val Asp Lys Phe Phe Pro Leu Ile Glu Val Asn Lys Val Tyr Tyr
 245 250 255
 Phe Ser Lys Gly Thr Leu Lys Ile Ala Asn Lys Gln Phe Thr Ala Val
 260 265 270
 Lys Asn Asp Tyr Glu Met Thr Phe Asn Asn Glu Thr Ser Val Met Pro
 275 280 285
 Cys Glu Asp Asp His His Leu Pro Thr Val Gln Phe Asp Phe Thr Gly
 290 295 300
 Ile Asp Asp Leu Glu Asn Lys Ser Lys Asp Ser Leu Val Asp Ile Ile
 305 310 315 320
 Gly Ile Cys Lys Ser Tyr Glu Asp Ala Thr Lys Ile Thr Val Arg Ser
 325 330 335
 Asn Asn Arg Glu Val Ala Lys Arg Asn Ile Tyr Leu Met Asp Thr Ser
 340 345 350
 Gly Lys Val Val Thr Ala Thr Leu Trp Gly Glu Asp Ala Asp Lys Phe
 355 360 365
 Asp Gly Ser Arg Gln Pro Val Leu Ala Ile Lys Gly Ala Arg Val Ser
 370 375 380
 Asp Phe Gly Gly Arg Ser Leu Ser Val Leu Ser Ser Ser Thr Ile Ile
 385 390 395 400
 Ala Asn Pro Asp Ile Pro Glu Ala Tyr Lys Leu Arg Gly Trp Phe Asp
 405 410 415
 Ala Glu Gly Gln Ala Leu Asp Gly Val Ser Ile Ser Asp Leu Lys Ser
 420 425 430
 Gly Gly Val Gly Gly Ser Asn Thr Asn Trp Lys Thr Leu Tyr Glu Val
 435 440 445
 Lys Ser Glu Asn Leu Gly Gln Gly Asp Lys Pro Asp Tyr Phe Ser Ser
 450 455 460
 Val Ala Thr Val Val Tyr Leu Arg Lys Glu Asn Cys Met Tyr Gln Ala
 465 470 475 480
 Cys Pro Thr Gln Asp Cys Asn Lys Lys Val Ile Asp Gln Gln Asn Gly
 485 490 495
 Leu Tyr Arg Cys Glu Lys Cys Asp Thr Glu Phe Pro Asn Phe Lys Tyr
 500 505 510
 Arg Met Ile Leu Ser Val Asn Ile Ala Asp Phe Gln Glu Asn Gln Trp
 515 520 525
 Val Thr Cys Phe Gln Glu Ser Ala Glu Ala Ile Leu Gly Gln Asn Ala

```

      530                      535                      540
Ala Tyr Leu Gly Glu Leu Lys Asp Lys Asn Glu Gln Ala Phe Glu Glu
545                      550                      555                      560
Val Phe Gln Asn Ala Asn Phe Arg Ser Phe Ile Phe Arg Val Arg Val
      565                      570                      575
Lys Val Glu Thr Tyr Asn Asp Glu Ser Arg Ile Lys Ala Thr Val Met
      580                      585                      590
Asp Val Lys Pro Val Asp Tyr Arg Glu Tyr Gly Arg Arg Leu Val Met
      595                      600                      605
Ser Ile Arg Arg Ser Ala Leu Met
      610                      615

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<210> 8
<211> 603
<212> PRT
<213> Drosophila melanogaster

```

```

      <400> 8
Met Val Leu Ala Ser Leu Ser Thr Gly Val Ile Ala Arg Ile Met His
  1                      5                      10                      15
Gly Glu Val Val Asp Ala Pro Val Leu Gln Ile Leu Ala Ile Lys Lys
      20                      25                      30
Ile Asn Ser Ala Ala Asp Ser Glu Arg Tyr Arg Ile Leu Ile Ser Asp
      35                      40                      45
Gly Lys Tyr Phe Asn Ser Tyr Ala Met Leu Ala Ser Gln Leu Asn Val
      50                      55                      60
Met Gln His Asn Gly Glu Leu Glu Glu Phe Thr Ile Val Gln Leu Asp
      65                      70                      75                      80
Lys Tyr Val Thr Ser Leu Val Gly Lys Asp Gly Ala Gly Lys Arg Val
      85                      90                      95
Leu Ile Ile Ser Glu Leu Thr Val Val Asn Pro Gly Ala Glu Val Lys
      100                      105                      110
Ser Lys Ile Gly Glu Pro Val Thr Tyr Glu Asn Ala Ala Lys Gln Asp
      115                      120                      125
Leu Ala Pro Lys Pro Ala Val Thr Ser Asn Ser Lys Pro Ile Ala Lys
      130                      135                      140
Lys Glu Pro Ser His Asn Asn Asn Asn Asn Ile Val Met Asn Ser Ser
      145                      150                      155                      160
Ile Asn Ser Gly Met Thr His Pro Ile Ser Ser Leu Ser Pro Tyr Gln
      165                      170                      175
Asn Lys Trp Val Ile Lys Ala Arg Val Thr Ser Lys Ser Gly Ile Arg
      180                      185                      190
Thr Trp Ser Asn Ala Arg Gly Glu Gly Lys Leu Phe Ser Met Asp Leu
      195                      200                      205
Met Asp Glu Ser Gly Glu Ile Arg Ala Thr Ala Phe Lys Glu Gln Cys
      210                      215                      220
Asp Lys Phe Tyr Asp Leu Ile Gln Val Asp Ser Val Tyr Tyr Ile Ser
      225                      230                      235                      240
Lys Cys Gln Leu Lys Pro Ala Asn Lys Gln Tyr Ser Ser Leu Asn Asn
      245                      250                      255
Ala Tyr Glu Met Thr Phe Ser Gly Glu Thr Val Val Gln Leu Cys Glu
      260                      265                      270
Asp Thr Asp Asp Asp Pro Ile Pro Glu Ile Lys Tyr Asn Leu Val Pro
      275                      280                      285
Ile Ser Asp Val Ser Gly Met Glu Asn Lys Ala Ala Val Asp Thr Ile
      290                      295                      300
Gly Ile Cys Lys Glu Val Gly Glu Leu Gln Ser Phe Val Ala Arg Thr

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```

305          310          315          320
Thr Asn Lys Glu Phe Lys Lys Arg Asp Ile Thr Leu Val Asp Met Ser
          325          330          335
Asn Ser Ala Ile Ser Leu Thr Leu Trp Gly Asp Asp Ala Val Asn Phe
          340          345          350
Asp Gly His Val Gln Pro Val Ile Leu Val Lys Gly Thr Arg Ile Asn
          355          360          365
Glu Phe Asn Gly Gly Lys Ser Leu Ser Leu Gly Gly Gly Ser Ile Met
          370          375          380
Lys Ile Asn Pro Asp Ile Pro Glu Ala His Lys Leu Arg Gly Trp Phe
385          390          395          400
Asp Asn Gly Gly Gly Asp Ser Val Ala Asn Met Val Ser Ala Arg Thr
          405          410          415
Gly Gly Gly Ser Phe Ser Thr Glu Trp Met Thr Leu Lys Asp Ala Arg
          420          425          430
Ala Arg Asn Leu Gly Ser Gly Asp Lys Pro Asp Tyr Phe Gln Cys Lys
          435          440          445
Ala Val Val His Ile Val Lys Gln Glu Asn Ala Phe Tyr Arg Ala Cys
          450          455          460
Pro Gln Ser Asp Cys Asn Lys Lys Val Val Asp Glu Gly Asn Asp Gln
465          470          475          480
Phe Arg Cys Glu Lys Cys Asn Ala Leu Phe Pro Asn Phe Lys Tyr Arg
          485          490          495
Leu Leu Ile Asn Met Ser Ile Gly Asp Trp Thr Ser Asn Arg Trp Val
          500          505          510
Ser Ser Phe Asn Glu Val Gly Glu Gln Leu Leu Gly His Thr Ser Gln
          515          520          525
Glu Val Gly Glu Ala Leu Glu Asn Asp Pro Ala Lys Ala Glu Gln Ile
          530          535          540
Phe Ser Ala Leu Asn Phe Thr Ser His Ile Phe Lys Leu Arg Cys Lys
545          550          555          560
Asn Glu Val Tyr Gly Asp Met Thr Arg Asn Lys Leu Thr Val Gln Ser
          565          570          575
Val Ala Pro Ile Asn His Lys Glu Tyr Asn Lys His Leu Leu Lys Glu
          580          585          590
Leu Gln Glu Leu Thr Gly Ile Gly Ser Ser Asn
          595          600

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```

<210> 9
<211> 609
<212> PRT
<213> Schizosaccharomyces pombe

```

```

<400> 9
Met Ala Glu Arg Leu Ser Val Gly Ala Leu Arg Ile Ile Asn Thr Ser
 1          5          10          15
Asp Ala Ser Ser Phe Pro Pro Asn Pro Ile Leu Gln Val Leu Thr Val
          20          25          30
Lys Glu Leu Asn Ser Asn Pro Thr Ser Gly Ala Pro Lys Arg Tyr Arg
          35          40          45
Val Val Leu Ser Asp Ser Ile Asn Tyr Ala Gln Ser Met Leu Ser Thr
          50          55          60
Gln Leu Asn His Leu Val Ala Glu Asn Lys Leu Gln Lys Gly Ala Phe
65          70          75          80
Val Gln Leu Thr Gln Phe Thr Val Asn Val Met Lys Glu Arg Lys Ile
          85          90          95
Leu Ile Val Leu Gly Leu Asn Val Leu Thr Glu Leu Gly Val Met Asp

```


17

```
<210> 10
<211> 621
<212> PRT
<213> Saccharomyces cerevisiae
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18

Gly Lys Lys Phe Asp Arg Arg Asp Ile Thr Ile Val Asp Asp Ser Gly
 340 345 350
 Phe Ser Ile Ser Val Gly Leu Trp Asn Gln Gln Ala Leu Asp Phe Asn
 355 360 365
 Leu Pro Glu Gly Ser Val Ala Ile Lys Gly Val Arg Val Thr Asp
 370 375 380
 Phe Gly Gly Lys Ser Leu Ser Met Gly Phe Ser Ser Thr Leu Ile Pro
 385 390 395 400
 Asn Pro Glu Ile Pro Glu Ala Tyr Ala Leu Lys Gly Trp Tyr Asp Ser
 405 410 415
 Lys Gly Arg Asn Ala Asn Phe Ile Thr Leu Lys Gln Glu Pro Gly Met
 420 425 430
 Gly Gly Gln Ser Ala Ala Ser Leu Thr Lys Phe Ile Ala Gln Arg Ile
 435 440 445
 Thr Ile Ala Arg Ala Gln Ala Glu Asn Leu Gly Arg Ser Glu Lys Gly
 450 455 460
 Asp Phe Phe Ser Val Lys Ala Ala Ile Ser Phe Leu Lys Val Asp Asn
 465 470 475 480
 Phe Ala Tyr Pro Ala Cys Ser Asn Glu Asn Cys Asn Lys Lys Val Leu
 485 490 495
 Glu Gln Pro Asp Gly Thr Trp Arg Cys Glu Lys Cys Asp Thr Asn Asn
 500 505 510
 Ala Arg Pro Asn Trp Arg Tyr Ile Leu Thr Ile Ser Ile Ile Asp Glu
 515 520 525
 Thr Asn Gln Leu Trp Leu Thr Leu Phe Asp Asp Gln Ala Lys Gln Leu
 530 535 540
 Leu Gly Val Asp Ala Asn Thr Leu Met Ser Leu Lys Glu Glu Asp Pro
 545 550 555 560
 Asn Glu Phe Thr Lys Ile Thr Gln Ser Ile Gln Met Asn Glu Tyr Asp
 565 570 575
 Phe Arg Ile Arg Ala Arg Glu Asp Thr Tyr Asn Asp Gln Ser Arg Ile
 580 585 590
 Arg Tyr Thr Val Ala Asn Leu His Ser Leu Asn Tyr Arg Ala Glu Ala
 595 600 605
 Asp Tyr Leu Ala Asp Glu Leu Ser Lys Ala Leu Leu Ala
 610 615 620

<210> 11

<211> 1124

<212> DNA

<213> Zea mays

<220>

<221> misc_feature

<222> (0)...(0)

<223> Maize RPA Middle Subunit Homologue-1

<221> CDS

<222> (76)...(894)

<400> 11

tcgacccacg cgtccgatacc tcccattctgc gcacccgcaa gcctattcgc cgcacctcct 60
 caggtgaccg ggaag atg atg ccg ttg agc caa acc gac ttc tcg ccg tcg 111
 Met Met Pro Leu Ser Gln Thr Asp Phe Ser Pro Ser
 1 5 10
 cag ttc acc tcc tcc cag aat gcc gcc gcc gac tcc acc acg cct tcc 159

Gln Phe Thr Ser Ser Gln Asn Ala Ala Ala Asp Ser Thr Thr Pro Ser	
15 20 25	
aag atg cgc ggc gcg tcc agc acc atg ccg ctc acc gtg aag cag gtc	207
Lys Met Arg Gly Ala Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val	
30 35 40	
gtc gac gcg cag cag tct ggc acg ggc gag aag ggc gct ccg ttc atc	255
Val Asp Ala Gln Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile	
45 50 55 60	
gtc aat ggc gtc gag atg gct aac att cga ctt gtg ggg atg gtc aat	303
Val Asn Gly Val Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn	
65 70 75	
gcc aag gtg gag cgg acg acc gat gtg acc ttc acg ctc gac gat ggc	351
Ala Lys Val Glu Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly	
80 85 90	
acc ggc cgc ctc gat ttc atc aga tgg gtg aat gat gct tca gat tct	399
Thr Gly Arg Leu Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser	
95 100 105	
ttt gaa act gct gct att cag aat ggt atg tac att gcg gtc att gga	447
Phe Glu Thr Ala Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly	
110 115 120	
agc ctc aag gga ctg caa gag agg aag cgt gct act gct ttc tca atc	495
Ser Leu Lys Gly Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile	
125 130 135 140	
agg cct ata acc gat ttc aat gag gtt acg ctg cat ttc att cag tgt	543
Arg Pro Ile Thr Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys	
145 150 155	
gtt cgg atg cat ata gag aac att gaa tta aag gct ggc agt cct gca	591
Val Arg Met His Ile Glu Asn Ile Glu Leu Lys Ala Gly Ser Pro Ala	
160 165 170	
cga atc agt tct tct atg gga gtg tca ttc tca aat gga ttc agt gaa	639
Arg Ile Ser Ser Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu	
175 180 185	
tca agc aca ccg aca tct ttg aaa tcc agt ccc gca ccg gtg acc agc	687
Ser Ser Thr Pro Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser	
190 195 200	
ggg tca tcc gat act gat ctg cac acg cag gtc ctg aat ttt ttt aat	735
Gly Ser Ser Asp Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn	
205 210 215 220	
gaa cca gcg aac ctc gag agt gag cat ggg gtg cac gtt gat gaa gta	783
Glu Pro Ala Asn Leu Glu Ser Glu His Gly Val His Val Asp Glu Val	
225 230 235	
ctc aag cgg ttc aaa ctt ttg ccg aag aag cag atc acg gat gct att	831
Leu Lys Arg Phe Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile	

240 245 250
 gat tac aat atg gac tcg ggg cgt ctt tac tca aca att gat gaa ttc 879
 Asp Tyr Asn Met Asp Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe
 255 260 265

cac tac aag gca act taaccgattt gaaggccagc ctgctggaaa tggcagagga 934
 His Tyr Lys Ala Thr
 270

ctaagtatca cttgtactaa accaaagtct ggaaatgtca tgttgtgtca tgaaatgcat 994
 gggttggttta tggaaacatt tatatcttgt atcaactagt tgatttgtat ctctgtgtcaa 1054
 cttaatgact gagccaagaa aaggaagatg tagaggccga cagaaaaaaaa aaaaaaaaaa 1114
 aaaaaaaaaa 1124

<210> 12
 <211> 273
 <212> PRT
 <213> Zea mays

<400> 12
 Met Met Pro Leu Ser Gln Thr Asp Phe Ser Pro Ser Gln Phe Thr Ser
 1 5 10 15
 Ser Gln Asn Ala Ala Ala Asp Ser Thr Thr Pro Ser Lys Met Arg Gly
 20 25 30
 Ala Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val Val Asp Ala Gln
 35 40 45
 Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile Val Asn Gly Val
 50 55 60
 Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu
 65 70 75 80
 Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu
 85 90 95
 Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala
 100 105 110
 Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly
 115 120 125
 Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr
 130 135 140
 Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His
 145 150 155 160
 Ile Glu Asn Ile Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Ser Ser
 165 170 175
 Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro
 180 185 190
 Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp
 195 200 205
 Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn
 210 215 220
 Leu Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe
 225 230 235 240
 Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met
 245 250 255
 Asp Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe His Tyr Lys Ala
 260 265 270
 Thr

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<220>
<221> misc_feature
<222> (0)...(0)
<223> Maize RPA Middle Subunit Homologue-2 and 3

<221> CDS
<222> (37)...(855)
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22

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aag gct ggc agt cct gca cga atc agt tct tct atg gga gtg tca ttc	582																			
Lys Ala Gly Ser Pro Ala Arg Ile Ser Ser Ser Met Gly Val Ser Phe																				
170	175	180																		
tca aat gga ttc agt gaa tca agc aca ccg aca tct ttg aaa tcc agt	630																			
Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro Thr Ser Leu Lys Ser Ser																				
185	190	195																		
ccc gca ccg gtg acc agc ggg tca tcc gat act gat ctg cac acg cag	678																			
Pro Ala Pro Val Thr Ser Gly Ser Ser Asp Thr Asp Leu His Thr Gln																				
200	205	210																		
gtc ctg aat ttt ttt aat gaa cca gcg aac ctc gag agt gag cat ggg	726																			
Val Leu Asn Phe Phe Asn Glu Pro Ala Asn Leu Glu Ser Glu His Gly																				
215	220	225	230																	
gtg cac gtt gat gaa gta ctc aag cgg ttc aaa ctt ttg ccg aag aag	774																			
Val His Val Asp Glu Val Leu Lys Arg Phe Lys Leu Leu Pro Lys Lys																				
235	240	245																		
cag atc acg gat gct att gat tac aat atg gac tcg ggg cgt ctt tac	822																			
Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met Asp Ser Gly Arg Leu Tyr																				
250	255	260																		
tca aca att gat gaa ttc cac tac aag gca act taaccgattt gaaggccagc	875																			
Ser Thr Ile Asp Glu Phe His Tyr Lys Ala Thr																				
265	270																			
ctgctggaaa tggcagagga ctaagtatca cttgtactaa accaaagtct ggaaatgtca	935																			
tggtgtgtca tgaatgcat ggttgggtta tggaaacaaa aaaa	979																			

<210> 14
 <211> 273
 <212> PRT
 <213> Zea mays

<400> 14
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 Ala Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val Val Asp Ala Gln
 35 40 45
 Gln Ser Gly Thr Gly Asp Lys Gly Ala Pro Phe Ile Val Asn Gly Val
 50 55 60
 Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu
 65 70 75 80
 Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu
 85 90 95
 Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala
 100 105 110
 Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly
 115 120 125
 Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr
 130 135 140

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Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His
145                      150                      155                      160
Ile Glu Asn Ile Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Ser Ser
                      165                      170                      175
Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro
                      180                      185                      190
Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp
                      195                      200                      205
Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn
                      210                      215                      220
Leu Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe
225                      230                      235                      240
Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met
                      245                      250                      255
Asp Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe His Tyr Lys Ala
                      260                      265                      270
Thr

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<210> 15
<211> 1051
<212> DNA
<213> Zea mays

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<220>
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<222> (0)...(0)
<223> Maize RPA Middle Subunit Homologue-4

<221> CDS
<222> (76)...(894)

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                Met Met Pro Leu Ser Gln Thr Asp Phe Ser Pro Ser
                  1                      5                      10

cag ttc acc tcc tcc cag aat gcc gcc gcc gac tcc acc acg cct tcc      159
Gln Phe Thr Ser Ser Gln Asn Ala Ala Ala Asp Ser Thr Thr Pro Ser
                  15                      20                      25

aag atg cgc ggc gcg tcc agc acc atg ccg ctc acc gtg aag cag gtc      207
Lys Met Arg Gly Ala Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val
                  30                      35                      40

gtc gac gcg cag cag tct ggc acg ggc gag aag ggc gct ccg ttc atc      255
Val Asp Ala Gln Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile
                  45                      50                      55                      60

gtc aat ggc gtc gag atg gct aac att cga ctt gtg ggg atg gtc aat      303
Val Asn Gly Val Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn
                  65                      70                      75

gcc aag gtg gag cgg acg acc gat gtg acc ttc acg ctc gac gat ggc      351
Ala Lys Val Glu Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly
                  80                      85                      90

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acc ggc cgc ctc gat ttc atc aga tgg gtg aat gat gct tca gat tct      399
Thr Gly Arg Leu Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser
      95                      100                      105

ttt gaa act gct gct att cag aat ggt atg tac att gcg gtc att gga      447
Phe Glu Thr Ala Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly
      110                      115                      120

agc ctc aag gga ctg caa gag agg aag cgt gct act gct ttc tca atc      495
Ser Leu Lys Gly Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile
      125                      130                      135                      140

agg cct ata acc gat ttc aat gag gtt acg ctg cat ttc att cag tgt      543
Arg Pro Ile Thr Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys
      145                      150                      155

gtt cgg atg cat ata gag aac act gaa tta aag gct ggc agt cct gca      591
Val Arg Met His Ile Glu Asn Thr Glu Leu Lys Ala Gly Ser Pro Ala
      160                      165                      170

cga atc aat tct tct atg gga gtg tca ttc tca aat gga ttc agt gaa      639
Arg Ile Asn Ser Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu
      175                      180                      185

tca agc aca ccg aca tct ttg aaa tcc agt ccc gca ccg gtg acc agc      687
Ser Ser Thr Pro Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser
      190                      195                      200

ggg tca tcc gat act gat ctg cac acg cag gtc ctg aat ttt ttt aat      735
Gly Ser Ser Asp Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn
      205                      210                      215                      220

gaa cca gcg aac ctc gag agt gag cat ggg gtg cac gtt gat gaa gta      783
Glu Pro Ala Asn Leu Glu Ser Glu His Gly Val His Val Asp Glu Val
      225                      230                      235

ctc aag cgg ttc aaa ctt ttg ccg aag aag cag atc acg gat gct att      831
Leu Lys Arg Phe Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile
      240                      245                      250

gat tac aat atg gac tcg ggg cgt ctt tac tca aca att gat gaa ttc      879
Asp Tyr Asn Met Asp Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe
      255                      260                      265

cac tac aag gca act taaccgattt gaaggtcagc ctgctggaaa tggcagagga      934
His Tyr Lys Ala Thr
      270

ctaagtatca cttgtactaa accaaagtct ggaaatgtca tgttgtgtca tgaaatgcat      994
ggtttggttta tggaacaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa      1051

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<210> 16
<211> 273
<212> PRT
<213> Zea mays

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20      25      30
Ala Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val Val Asp Ala Gln
35      40      45
Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile Val Asn Gly Val
50      55      60
Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu
65      70      75      80
Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu
85      90      95
Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala
100     105     110
Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly
115     120     125
Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr
130     135     140
Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His
145     150     155     160
Ile Glu Asn Thr Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Asn Ser
165     170     175
Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro
180     185     190
Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp
195     200     205
Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn
210     215     220
Leu Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe
225     230     235     240
Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met
245     250     255
Asp Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe His Tyr Lys Ala
260     265     270
Thr

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<210> 17
<211> 1087
<212> DNA
<213> Zea mays

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<220>
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<222> (0)...(0)
<223> Maize RPA Middle Subunit Homologue-5

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<221> CDS
<222> (91)...(1044)

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ttcgcgcgac ctcctcaggt gaccgggaag atg atg ccg ttg agc caa acc gac      114
Met Met Pro Leu Ser Gln Thr Asp
1      5

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ttc tcg ccg tcg cag ttc acc tcc tcc cag aat gcc gcc gcc gac tcc Phe Ser Pro Ser Gln Phe Thr Ser Ser Gln Asn Ala Ala Ala Asp Ser 10 15 20	162
acc acg cct tcc aag atg cgc ggc gcg tcc agc acc atg ccg ctc acc Thr Thr Pro Ser Lys Met Arg Gly Ala Ser Ser Thr Met Pro Leu Thr 25 30 35 40	210
gtg aag car gtc gtc gac gcg cag cag tct gcc acg gcc gag aag gcc Val Lys Xaa Val Val Asp Ala Gln Gln Ser Gly Thr Gly Glu Lys Gly 45 50 55	258
gct ccg ttc atc gtc aat gcc gtc gag atg gct aac att cga ctt gtg Ala Pro Phe Ile Val Asn Gly Val Glu Met Ala Asn Ile Arg Leu Val 60 65 70	306
ggg atg gtc aat gcc aag gtg gag cgg acg acc gat gtg acc ttc acg Gly Met Val Asn Ala Lys Val Glu Arg Thr Thr Asp Val Thr Phe Thr 75 80 85	354
ctc gac gat gcc acc gcc cgc ctc gat ttc atc aga tgg gtg aat gat Leu Asp Asp Gly Thr Gly Arg Leu Asp Phe Ile Arg Trp Val Asn Asp 90 95 100	402
gct tca gat tct ttt gaa act gct gct att cag aat ggt atg tac att Ala Ser Asp Ser Phe Glu Thr Ala Ala Ile Gln Asn Gly Met Tyr Ile 105 110 115 120	450
gcg gtc att gga agc ctc aag gga ctg caa gag agg aag cgt gct act Ala Val Ile Gly Ser Leu Lys Gly Leu Gln Glu Arg Lys Arg Ala Thr 125 130 135	498
gct ttc tca atc agg cct ata acc gat ttc aat gag gtt acg ctg cat Ala Phe Ser Ile Arg Pro Ile Thr Asp Phe Asn Glu Val Thr Leu His 140 145 150	546
ttc att cag tgt gtt cgg atg cat ata gag aac act gaa tta aag gct Phe Ile Gln Cys Val Arg Met His Ile Glu Asn Thr Glu Leu Lys Ala 155 160 165	594
ggc agt cct gca cga atc aat tct tct atg gga gtg tca ttc tca aat Gly Ser Pro Ala Arg Ile Asn Ser Ser Met Gly Val Ser Phe Ser Asn 170 175 180	642
gga ttc agt gaa tca agc aca ccg aca tct ttg aaa tcc agt ccc gca Gly Phe Ser Glu Ser Ser Thr Pro Thr Ser Leu Lys Ser Ser Pro Ala 185 190 195 200	690
ccg gtg acc agc ggg tca tcc gat act gat ctg cac acg cag gtc ctg Pro Val Thr Ser Gly Ser Ser Asp Thr Asp Leu His Thr Gln Val Leu 205 210 215	738
aat ttt ttt aat gaa cca gcg aac ctc gag agt gag cat ggg gtg cac Asn Phe Phe Asn Glu Pro Ala Asn Leu Glu Ser Glu His Gly Val His 220 225 230	786
gtt gat gaa gta ctc aag cgg ttc aac ttt tgc cga aga agc aga tca	834

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Val Asp Glu Val Leu Lys Arg Phe Asn Phe Cys Arg Arg Ser Arg Ser
    235                240                245

cgg atg cta ttg att aca ata tgg act cgg ggc gtc ttt act caa caa      882
Arg Met Leu Leu Ile Thr Ile Trp Thr Arg Gly Val Phe Thr Gln Gln
    250                255                260

ttg atg aat tcc act aca agg caa ctt aac cga ttt gaa ggt cag cct      930
Leu Met Asn Ser Thr Thr Arg Gln Leu Asn Arg Phe Glu Gly Gln Pro
    265                270                275                280

gct gga aat ggc aga gga cta agt atc act tgt act aaa cca aag tct      978
Ala Gly Asn Gly Arg Gly Leu Ser Ile Thr Cys Thr Lys Pro Lys Ser
    285                290                295

gga aat gtc atg ttg tgt cat gaa atg cat ggt tgg ttt atg gaa aca      1026
Gly Asn Val Met Leu Cys His Glu Met His Gly Trp Phe Met Glu Thr
    300                305                310

ttt ata tct tgt atc aac tagttgattt gtatctcttg tgtcaaaaaa      1074
Phe Ile Ser Cys Ile Asn
    315

aaaaaaaaaa aaa      1087

<210> 18
<211> 318
<212> PRT
<213> Zea mays

<220>
<221> VARIANT
<222> (1)...(318)
<223> Xaa = Any Amino Acid

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 20          25          30
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 35          40          45
Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile Val Asn Gly Val
 50          55          60
Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu
 65          70          75          80
Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu
 85          90          95
Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala
100          105          110
Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly
115          120          125
Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr
130          135          140
Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His
145          150          155          160
Ile Glu Asn Thr Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Asn Ser

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                165                170                175
Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro
                180                185                190
Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp
                195                200                205
Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn
                210                215                220
Leu Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe
                225                230                235                240
Asn Phe Cys Arg Arg Ser Arg Ser Arg Met Leu Leu Ile Thr Ile Trp
                245                250                255
Thr Arg Gly Val Phe Thr Gln Gln Leu Met Asn Ser Thr Thr Arg Gln
                260                265                270
Leu Asn Arg Phe Glu Gly Gln Pro Ala Gly Asn Gly Arg Gly Leu Ser
                275                280                285
Ile Thr Cys Thr Lys Pro Lys Ser Gly Asn Val Met Leu Cys His Glu
                290                295                300
Met His Gly Trp Phe Met Glu Thr Phe Ile Ser Cys Ile Asn
305                310                315

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<210> 19
<211> 1074
<212> DNA
<213> Zea mays

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<220>
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<222> (0)...(0)
<223> Maize RPA Middle Subunit Homologue-6

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<221> CDS
<222> (55)...(873)

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atg ccg ttg agc caa acc gac ttc tcg ccg tcg cag ttc acc tcc tcc      105
Met Pro Leu Ser Gln Thr Asp Phe Ser Pro Ser Gln Phe Thr Ser Ser
                    5                      10                      15

cag aat gcc gcc gcc gac tcc acc acg cct tcc aag atg cgc ggc gcg      153
Gln Asn Ala Ala Ala Asp Ser Thr Thr Pro Ser Lys Met Arg Gly Ala
                20                      25                      30

tcc agc acc atg ccg ctc acc gtg aag cag gtc gtc gac gcg cag cag      201
Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val Val Asp Ala Gln Gln
                35                      40                      45

tct ggc acg ggc gag aag ggc gct ccg ttc atc gtc aat ggc gtc gag      249
Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile Val Asn Gly Val Glu
                50                      55                      60                      65

atg gct aac att cga ctt gtg ggg atg gtc aat gcc aag gtg gag cgg      297
Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu Arg
                70                      75                      80

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acg acc gat gtg acc ttc acg ctc gac gat ggc acc ggc cgc ctc gat      345
Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu Asp
      85                      90                      95

ttc atc aga tgg gtg aat gat gct tca gat tct ttt gaa act gct gct      393
Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala Ala
      100                      105                      110

att cag aat ggt atg tac att gcg gtc att gga agc ctc aag gga ctg      441
Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly Leu
      115                      120                      125

caa gag agg aag cgt gct act gct ttc tca atc agg cct ata acc gat      489
Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr Asp
      130                      135                      140                      145

ttc aat gag gtt acg ctg cat ttc att cag tgt gtt cgg atg cat ata      537
Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His Ile
      150                      155                      160

gag aac act gaa tta aag gct ggc agt cct gca cga atc aat tct tct      585
Glu Asn Thr Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Asn Ser Ser
      165                      170                      175

atg gga gtg tca ttc tca aat gga ttc agt gaa tca agc aca ccg aca      633
Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro Thr
      180                      185                      190

tct ttg aaa tcc agt ccc gca ccg gtg acc agc ggg tca tcc gat act      681
Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp Thr
      195                      200                      205

gat ctg cac acg cag gtc ctg aat ttt ttt aat gaa cca gcg aac ctc      729
Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn Leu
      210                      215                      220                      225

gag agt gag cat ggg gtg cac gtt gat gaa gta ctc aag cgg ttc aaa      777
Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe Lys
      230                      235                      240

ctt ttg ccg aag aag cag atc acg gat gct att gat tac aat atg gac      825
Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met Asp
      245                      250                      255

tcg ggg cgt ctt tac tca aca att gat gaa ttc cac tac aag gca act      873
Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe His Tyr Lys Ala Thr
      260                      265                      270

taaccgattt gaaggtcagc ctgctggaaa tggcagagga ctaagtatca cttgtactaa      933
accaaagtct ggaaatgtca tggtgtgtca tgaaatgcat gggttggttta tggaacatt      993
tatactctgt atcaactagt tgatttgtat ctcttgtgtc aacttaatga ctgagccaac      1053
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<210> 20
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<213> Zea mays

<400> 20

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Ala Ser Ser Thr Met Pro Leu Thr Val Lys Gln Val Val Asp Ala Gln
 35          40          45
Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile Val Asn Gly Val
 50          55          60
Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu
 65          70          75          80
Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu
 85          90          95
Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala
 100          105          110
Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly
 115          120          125
Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr
 130          135          140
Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His
 145          150          155          160
Ile Glu Asn Thr Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Asn Ser
 165          170          175
Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro
 180          185          190
Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp
 195          200          205
Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn
 210          215          220
Leu Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe
 225          230          235          240
Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met
 245          250          255
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 260          265          270
Thr

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<210> 21

<211> 1231

<212> DNA

<213> Zea mays

<220>

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<222> (0)...(0)

<223> Maize RPA Middle Subunit Homologue-7

<221> CDS

<222> (85)...(903)

<400> 21

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Met Met Pro Leu Ser Gln Thr Asp Phe

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32

gat gaa gta ctc aag cgg ttc aaa ctt ttg ccg aag aag cag atc acg 831
 Asp Glu Val Leu Lys Arg Phe Lys Leu Leu Pro Lys Lys Gln Ile Thr
 235 240 245

 gat gct att gat tac aat atg gac tcg ggg cgt ctt tac tca aca att 879
 Asp Ala Ile Asp Tyr Asn Met Asp Ser Gly Arg Leu Tyr Ser Thr Ile
 250 255 260 265

 gat gaa ttc cac tac aag gca act taaccgattt gaaggtcagc ctgctggaaa 933
 Asp, Glu Phe His Tyr Lys Ala Thr
 270

 tggcagagga ctaagtatca cttgtactaa accaaagtct ggaaatgtca tgttgtgtca 993
 tgaaatgcat ggttggttta tggaaacatt tatatcttgt atcaactagt tgatttgtat 1053
 ctcttgtgtc aacttaatga ctgagccaac aaaaggaaga tgtagaggca gacagacatt 1113
 tgtagattgg ctgatatgctg attcgggtag ctggtccaat tgcaatctgg ggccaataa 1173
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<210> 22
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 <212> PRT
 <213> Zea mays

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 35 40 45
 Gln Ser Gly Thr Gly Glu Lys Gly Ala Pro Phe Ile Val Asn Gly Val
 50 55 60
 Glu Met Ala Asn Ile Arg Leu Val Gly Met Val Asn Ala Lys Val Glu
 65 70 75 80
 Arg Thr Thr Asp Val Thr Phe Thr Leu Asp Asp Gly Thr Gly Arg Leu
 85 90 95
 Asp Phe Ile Arg Trp Val Asn Asp Ala Ser Asp Ser Phe Glu Thr Ala
 100 105 110
 Ala Ile Gln Asn Gly Met Tyr Ile Ala Val Ile Gly Ser Leu Lys Gly
 115 120 125
 Leu Gln Glu Arg Lys Arg Ala Thr Ala Phe Ser Ile Arg Pro Ile Thr
 130 135 140
 Asp Phe Asn Glu Val Thr Leu His Phe Ile Gln Cys Val Arg Met His
 145 150 155 160
 Ile Glu Asn Thr Glu Leu Lys Ala Gly Ser Pro Ala Arg Ile Asn Ser
 165 170 175
 Ser Met Gly Val Ser Phe Ser Asn Gly Phe Ser Glu Ser Ser Thr Pro
 180 185 190
 Thr Ser Leu Lys Ser Ser Pro Ala Pro Val Thr Ser Gly Ser Ser Asp
 195 200 205
 Thr Asp Leu His Thr Gln Val Leu Asn Phe Phe Asn Glu Pro Ala Asn
 210 215 220
 Leu Glu Ser Glu His Gly Val His Val Asp Glu Val Leu Lys Arg Phe
 225 230 235 240
 Lys Leu Leu Pro Lys Lys Gln Ile Thr Asp Ala Ile Asp Tyr Asn Met
 245 250 255

Asp Ser Gly Arg Leu Tyr Ser Thr Ile Asp Glu Phe His Tyr Lys Ala
260 265 270
Thr

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/21277

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/82 C12N15/11 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>VAN DER KNAAP, E., ET AL: "Expression of an ortholog of replication protein A1 (RPA1) is induced by gibberellin in deepwater rice"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 94, September 1997 (1997-09), pages 9979-9983, XP002131706 WASHINGTON US the whole document</p> <p>-& VAN DER KNAAP, E., ET AL.: "Oryza sativa replication protein A1 (Os-RPA1) mRNA, complete cds"</p> <p>EMBL ACCESSION NO:AF009179, 18 July 1997 (1997-07-18), XP002131707</p> <p style="text-align: center;">-/-</p>	1-3

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentean 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

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Maddox, A

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